TEMPORAL AND SPATIAL VARIABILITY IN "TETRACOTYLE" TYPE METACERCARIAE INFECTION IN THE SOUTH AFRICAN SARDINE, *SARDINOPS SAGAX*

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PLAGIARISM DECLARATION

I know the meaning of plagiarism and declare that all of the work in the dissertation, save for that which is properly acknowledged, is my own. I have not allowed, and will not allow anyone to copy my work.

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ABSTRACT:

Spatial and temporal variation of "tetracotyle" type metacercariae infection in the eyes of the South African sardine, *Sardinops sagax,* was examined to elucidate the potential use of this parasite as a biological tag, and to test the hypothesis that the sardine population is divided into discrete western and southern subpopulations or stocks. Adult *S. sagax* specimens of 15 to 22 cm caudal length were collected monthly from five commercial fishery landing harbours to the west (St. Helena and Gans Bay) and to the east (Mossel Bay and Port Elizabeth) of Cape Agulhas in 2011 and 2012. Samples were preserved whole in 70% ethanol, or frozen, and then bagged and labelled. Fish were measured (caudal length in cm), sexed and dissected and summary statistics on the infection by "tetracotyle" type metacercariae in their eyes were recorded. Prevalence of infection (%), infection intensity and parasite abundance were analysed seasonally, over a period of 18 months, in fish caught to the west of Cape Agulhas and presumed to be part of the putative western stock of sardine, and in fish caught to the east of Cape Agulhas presumed to be part of the putative southern stock. Generalised linear models were used to model these three indices as dependent on stock, season, year and caudal length, where a binomial distribution was assumed for prevalence and a negative binomial distribution was assumed for infection intensity and parasite abundance. All factors contributed significantly to all models, but it was found that stock was the most significant contributor to the deviance seen in prevalence (%) and parasite abundance, and was the second most important contributor to the deviance seen in infection intensity. Fish to the west of Cape Agulhas were found to have significantly higher parasite loads in comparison to fish from the east of Cape Agulhas ($p< 0.001$). Season was the second most significant contributor to the deviance seen in prevalence and abundance, and was the most important contributor to the deviance seen in infection intensity, indicating that a seasonal signal was present. This seasonal signal was slightly delayed in fish from the

putative southern stock in comparison to those from the putative western stock. Interannual differences in infection rates were also observed, being higher in 2012 compared to 2011. These results suggest that "tetracotyle" type metacercariae can be used as a biological tag in stock discrimination studies and that, despite temporal variability, the clear spatial difference in the distribution of infection of *S. sagax* by "tetracotyle" type metacercariae supports the hypothesis of western and southern stocks of sardine off the coast of South Africa.

CHAPTER 1: Introduction and Literature Review

Introduction:

Human population growth as well as the increase in technology and in the production and distribution of fish products, has resulted in the steady increase in the global consumption of fish and fish products since the 1960s (FAO, 2012). Because of this there has been a significant global increase in fishing effort which has resulted in many of the world's fisheries becoming fully-exploited or over-exploited (FAO, 2012). In combination with the effects of environmental changes, the overexploitation of many of the world's fish stocks has resulted in a major decline in their abundance. These stocks are therefore not considered to be at a population level where they can produce and maintain a maximum sustainable yield, and so there is uncertainty over their future sustainability (Baldwin *et al.,* 2012; FAO, 2012). To restore the abundance of stocks, and to ensure that sustainability is achieved, more careful and effective management plans are vital (FAO, 2012).

In order to effectively manage a fishery and successfully employ stock rebuilding strategies, the characteristics and possible stock structure of the population being managed needs to be known (Waldman, 2005). The notion of stock identification therefore becomes important as this allows for the understanding of how or why specific components of an exploited population are being targeted by the relative fishery (Baldwin *et al.,* 2012). This understanding will allow for the implementation of models that are more specific to the characteristics of the stock/s being exploited, and thus will allow for more effective management of the resource.

Commercially exploited fish stocks in the southeast Atlantic provide no exception to global trends, where, overall there has been a steady decline in catches since the 1970s (FAO, 2012). Changes in the population size and distribution of the South African sardine, *Sardinops* *sagax,* have been observed over the past several decades. This species occurs on the west, south and east coasts of South Africa, and is a short-lived, fast growing epipelagic fish that responds rapidly to environmentally induced changes (Department of Agriculture, Forestry and Fisheries, 2012). Several successive years of poor recruitment as well as high exploitation rates, predominantly off the west coast, have resulted in the decline in the abundance of sardine in South African waters over the past several years (Department of Agriculture, Forestry and Fisheries, 2012; FAO, 2012). Sardine are a major pelagic resource off the coast of South Africa, with high commercial value. The decline in their abundance is therefore of great commercial concern. Additionally, *S. sagax* is a major forage species, forming an important component of the food web (Branch *et al.,* 2010; Department of Agriculture, Forestry and Fisheries, 2012), and so its decline in abundance has a variety of far-reaching ecological effects.

Currently, the South African sardine stock is managed as a single population via an operational management procedure. This procedure uses input from biannual acoustic surveys to develop regulatory mechanisms, in terms of fishing quotas, on an annual basis (Barange *et al.,* 1999; Anon, 2004; Department of Agriculture, Forestry and Fisheries, 2012). In recent years however, this single stock approach to the management of the sardine fishery has come into question, where issues have arisen over the stock structure of the South African sardine. A variety of studies, using a range of different approaches, such as differences in meristics, morphometrics and life history characteristics, have hypothesised that there are in fact more than one stock of sardine off the Coast of South Africa. These studies have supported the occurrence of two stocks, to the west and east of Cape Agulhas, while a third stock may occur off the east coast of South Africa (van der Lingen, 2011). If the occurrence of these multiple stocks is true, then the multi-stock nature of the South African sardine will have to be incorporated into management, thus affecting management of the South African sardine fishery.

In addition to the use of meristics, morphometrics and life history characteristics, the use of parasites as biological tags is another approach that can be used in the identification of discrete fish stocks. It is based on the idea that a fish can only become infected with a particular parasite when it is within the distribution of that parasite- the endemic area. If an infected fish is not within the endemic area it can be assumed that it was once there (MacKenzie & Abaunza, 1998). The parasite distribution must therefore be smaller than the host range for it to be useful as a biological tag. Other criteria for a successful biological tag include the easy detection and identification of the parasite, no significant impact on the behaviour of the host, no selective mortality of the host, significantly different levels of infection of the host within the study area, a relatively long life span, a single host life cycle and a stable infection of the host over time- there cannot be seasonality in the infection, or if there is, it needs to be understood (Kabata, 1963; Sindermann, 1983; MacKenzie, 1983, 1987; Williams *et al.,* 1992, cited in MacKenzie & Abaunza, 1998).

Reed *et al.* (2012) conducted a study on sardine in South African waters with the aim of identifying possible biological tags. They found spatial variation in a digenean parasite of the "tetracotyle" type, found in the eyes of the fish, and concluded that this species had the most potential as a biological tag in order to differentiate different stocks of the South African sardine. There was much higher prevalence of the parasite in fish to the west of Cape Agulhas in comparison to fish from the east of Cape Agulhas. These findings supported the multiple stock hypothesis. Subsequent work conducted by van der Lingen (2011), using more fish from a larger area, confirmed the discontinuous distribution of the "tetracotyle" parasite around the South African coast. The current study complemented the initial studies by focussing particularly on seasonal patterns of the "tetracotyle" parasite in the putative western and southern sardine stocks, in order to gain more insight into the possibility of its use as a biological tag.

Sardinops sagax:

Biology and taxonomy of Sardinops sagax in southern African waters:

The sardine, *Sardinops sagax* is a small epipelagic species that is one of 13 species in southern Africa which falls into the family Clupeidae (Beckley & van der Lingen, 1999). Globally, Clupeoids form the most important group of commercially exploited pelagic species, where they are found to dominate many of the world's major upwelling systems (van der Lingen, 2002), and contribute a large part to annual global catches. The monotypic *Sardinops* genus is found within this family, where the species *Sardinops sagax* is recognised (Beckley & van der Lingen, 1999; Checkley *et al.,* 2009). It was once thought that there were in fact five species that occurred within the genus *Sardinops*, but after a comprehensive study on systematics, distribution, stock structure and zoogeography, Parrish *et al.* (1989) cited in Beckley & van der Lingen (1999) concluded that there was not a distinct enough difference between the different populations to consider them to be different species or subspecies. *Sardinops sagax* is therefore the commercially important sardine species found within South African waters- primarily within the Benguela upwelling system, where this forms one of the five major areas that this species is harvested (Beckley & van der Lingen, 1999; Checkley *et al.,* 2009; Department of Agriculture, Forestry and Fisheries*,* 2012). Catch trends of *S. sagax* in all five of these areas have shown long-term fluctuations, characteristic of many small pelagic species (Checkley *et al.,* 2009).

Sardinops sagax is a filter-feeding omnivore that thrives in productive regions such as the Benguela upwelling system present off the west coast of Southern Africa. It is a cooler water species that inhabits temperate coastal and shelf waters, such as those shallower waters around southern Africa. It forms an essential component of pelagic food webs where many forage species are dependent on it for survival (Checkley *et al.,* 2009; Branch *et al.,* 2010; Department of Agriculture, Forestry and Fisheries*,* 2012). *Sardinops sagax* is a short-lived, fast-growing species that is capable of producing thousands of eggs per spawning. Additionally, studies done by le Clus (1977, 1979a, 1979b) cited in Beckley & van der Lingen (1999) on oocyte development of sardine off the coast of Namibia suggests that they are able to spawn more than once a year. Due to their r-selected characteristics and their planktivorous diets, *S. sagax* respond rapidly to environmental fluctuations (Checkley *et al.,* 2009). This means that under unfavourable environmental conditions, or when harvesting levels are too high, sardine populations can decline dramatically. However, when fishing pressure is appropriate and environmental conditions are optimal, due to their high fecundity, sardine populations have the capacity to increase rapidly and significantly (Department of Agriculture, Forestry and Fisheries*,* 2012). Once developed, *S. sagax* can assemble very large, dense shoals on which a variety of marine mammals, birds and fish predate (van der Lingen & Huggett, 2003; Martinez-Porchas *et al.,* 2009; Branch *et al.,* 2010; Department of Agriculture, Forestry and Fisheries*,* 2012).

The distribution of Sardinops sagax around southern Africa:

In southern Africa, the distribution of *S. sagax* ranges from southern Angola on the west coast of Africa, up to Richards Bay on the east coast (Beckley & van der Lingen, 1999; Checkley *et al.,* 2009; Branch *et al.,* 2010). Within this distribution, there appears to be a separation of the sardine population off the coast of Luderitz due to the presence of an intense upwelling cell causing a thermal barrier (Beckley & van der Lingen, 1999). Thus there is a distinct separation between the Namibian sardine population and the South African sardine population (Coetzee *et al.,* 2008).

The South African population, in which this study is primarily interested, extends southwards from the Orange River mouth in the south of Namibia all the way around South Africa, to Richards Bay (Beckley & van der Lingen, 1999). Within this population, it has been suggested by various authors that there may be a separation in the distribution of *S. sagax* off the South African coast, which may suggest that there is more than one stock of *S. sagax* that makes up the South African population. This will be discussed in more detail at a later stage.

Less is known about the recruitment, distribution and migration habits at the different life stages of the sardine in South African waters, in comparison to anchovy, but it is assumed that there is similarity with anchovy, *Engraulis encrasicolus,* for which patterns have been well established (Coetzee *et al.,* 2008). Additionally, hydro-acoustic surveys that have been run since 1984, have provided invaluable information on the sardine stock, that can be used to distinguish patterns of recruitment, distribution and migration (Barange *et al.,* 1999; Coetzee *et al.,* 2008; Department of Agriculture, Forestry and Fisheries, 2012). Many studies have shown sardine off the South African coast to show a clear size- or age-related distribution (Beckley & van der Lingen, 1999; van der Lingen & Huggett, 2003; Coetzee *et al.,* 2008).

Up to 1999, studies have shown that spawning adults predominantly occurred on the south west coast, on the western Agulhas Bank, where the concentration of 2 to 4 year old fish was highest during the peak in the sardine spawning season, from August through to March (Barange *et al.,* 1999; Beckley & van der Lingen, 1999; Coetzee *et al.,* 2008). Spawning was however also known to shift and to occur on the south coast relatively frequently, where, since 2001, large amounts of eggs have been found to the east of Cape Agulhas (van der Lingen *et al.,* 2006b; Coetzee *et al.,* 2008). This shift in spawning habitats is due to the fact that sardine are fairly robust in their choice of spawning conditions, and are less restricted by temperature than other small pelagic species (van der Lingen & Huggett, 2003). However, this variation in egg distribution was not always closely matched to the adult fish biomass patterns and the presence of 0 year old sardine recruits, as well as 1 year old fish, remained dominant on the west coast due to the transport of the larvae as plankton up the west coast by a jet current (Barange *et al.,* 1999; Coetzee *et al.,* 2008).

There is a migration of two to four year old fish up the east coast during winter- a phenomenon known as the annual sardine run- that is thought to be a seasonal reproductive migration undertaken by a genetically distinct subpopulation of sardine that spawn off the KwaZulu Natal coast during winter (Connell, 2010; Freon *et al.,* 2010). It is thought to be caused by the expansion of the preferred cooler water environment of sardines northwards, up the east coast, in the early winter months. Current reversals, upwelling of cool water on the narrow continental shelf, the presence of transient cyclonic eddies, small-scale physical processes and even pursuit by predators may cause the sardines to congregate along the continental shelf, resulting in the sardine run (Armstrong *et al.,* 1991; Roberts *et al.,* 2010).

In the 1980s and early 1990s, the majority of the sardine biomass was therefore located on the west coast, to the west of Cape Agulhas (Barange *et al.,* 1999). Acoustic surveys have however helped to identify that since 1999, there has been a shift in the distribution of sardine in South African waters, where there has been an increase in biomass to the east of Cape Agulhas causing the biomass to the east of Cape Agulhas to exceed that to the west (van der Lingen *et al.,* 2006b; Coetzee *et al.,* 2008; Department of Agriculture, Forestry and Fisheries, 2012). This shift in the distribution was subsequently followed by a number of successive years of poor recruitment, consequently resulting in lowered abundance levels and contributing to the already decreased abundance of sardine off the west coast (Department of

Agriculture, Forestry and Fisheries*,* 2012). Many reasons have been suggested for this, including increased exploitation of the sardine stocks on the west coast compared to the east coast, environmentally induced changes, and the increased success of south coast spawning with the local retention of eggs and larvae (Coetzee *et al.,* 2008).

This has economic and logistical implications because, due to the previous trends in sardine distribution, majority of the processing plants are present on the west coast of South Africa, resulting in the need for fishing vessels to travel further to catch sardine and the need to transport catches back from the east to the west in order for them to be processed (Coetzee *et al.,* 2008; Department of Agriculture, Forestry and Fisheries*,* 2012). The shift in abundance also has had a variety of ecological implications, where sardine predators off the west coast have had to change their diets to feed on other species, many of which have a lower energetic content, and predators also have to travel further in order to feed. These two factors combined have resulted in decreased breeding success of certain species such as the Cape Gannet *Morus capensis* (Department of Agriculture, Forestry and Fisheries*,* 2012).

The South African sardine fishery:

Sardines play a vital role in the commercial pelagic purse-seine fishery within South Africa, where fishing has been focussed on the west coast since the 1940s. The small pelagic fishery within South Africa is the largest in terms of volumes caught and the second highest in net value of all South African commercial fisheries (Agenbag *et al.,* 2003; van der Lingen & Huggett, 2003; Anon, 2004; Coetzee *et al.,* 2008; Department of Agriculture, Forestry and Fisheries*,* 2012). Along with anchovy, sardine form the basis of the pelagic resource in South African waters, and so are a major target species, but additionally, juvenile sardine are caught as by-catch in the anchovy-directed fishery. Sardines are targeted primarily for canning, human consumption and bait (Barange *et al.,* 1999; Agenbag *et al.,* 2003; Anon, 2004;

Coetzee *et al.,* 2008; Department of Agriculture, Forestry and Fisheries*,* 2012). Over the years, the sardine fishery has experienced severe fluctuations due to a variety of different reasons, and so because of the commercial importance of the species, management and maintenance of the stock is of primary importance (Anon, 2004; Department of Agriculture, Forestry and Fisheries*,* 2012). Because of this, there has been much research focussed on *S. sagax* in southern African waters over the last several decades (Beckley & van der Lingen, 1999).

Sardine biomass in South African waters was high in the 1950s and early 1960s, where catches peaked in 1962 at 410 000 t. This level of exploitation soon proved to be too high, and coupled with poor recruitment, it led first to the decline of catches and then the eventual collapse of the South African sardine fishery by the late 1960s (Agenbag *et al.,* 2003; Anon, 2004; Coetzee *et al.,* 2008; Department of Agriculture, Forestry and Fisheries*,* 2012).

Catches remained low throughout the 1970s and the early 1980s, where they declined to approximately 40 000 t (Anon, 2004; Coetzee *et al.,* 2008). In the late 1980s, following the introduction of the acoustic surveys, a stock-rebuilding strategy was implemented, which included the enforcement of a annual total allowable catch (TAC) which was based on the results obtained from the acoustic surveys that were run biannually (Coetzee *et al.,* 2008). Gradually, with the implementation of this conservative strategy, the sardine stocks began to recover and catches started to increase in the mid-1990s.

Between 2001 and 2005, sardine catches averaged more than 200 000 t annually due to an exceptional recruitment period, with a peak in catches in 2004 at 374 000 t (Coetzee *et al.,* 2008; Department of Agriculture, Forestry and Fisheries*,* 2012). The majority of the sardine caught during this period, were caught off the south coast (Department of Agriculture, Forestry and Fisheries*,* 2012). However, poor recruitment since 2004 has again led to the decline in sardine biomass and catches in South African waters since 2005 (Coetzee *et al.,* 2008; FAO, 2012; Department of Agriculture, Forestry and Fisheries, 2012). Records of the annual commercial sardine catch off the coast of South Africa are shown in Figure 1.1.

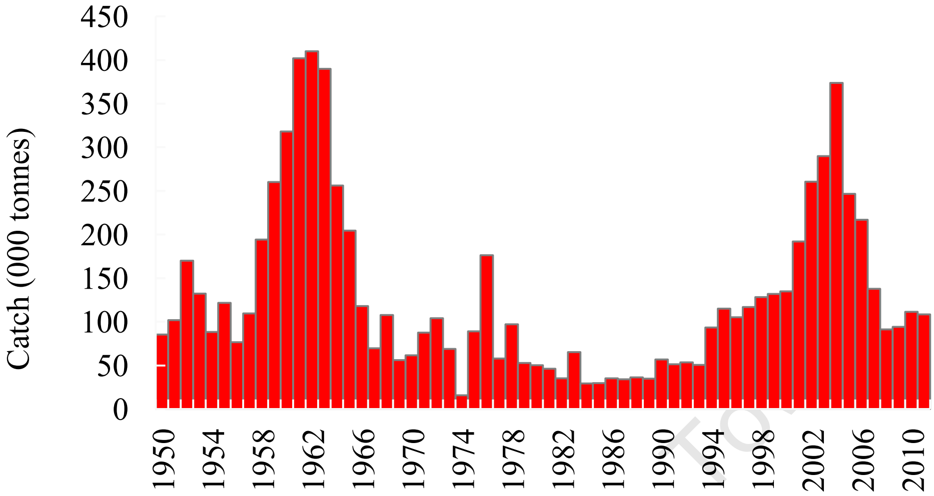

Figure 1.1: Annual commercial sardine catches off the coast of South Africa from 1950 to 2011.

Presently, the South African sardine stock is managed as one population via an operational management procedure that uses data from the biannual acoustic surveys to develop regulatory mechanisms, such as a TAC, each year (Barange *et al.,* 1999; Agenbag *et al.,* 2003; Anon, 2004; Department of Agriculture, Forestry and Fisheries, 2012). Due to the complicating factor of juvenile sardine being caught as by-catch in the anchovy directed fishery, the TAC for sardine has to be split into two: a sardine directed TAC and a by-catch TAC or a total allowable by-catch (TAB) (Agenbag *et al.,* 2003; Department of Agriculture, Forestry and Fisheries, 2012). More recently an ecological risk assessment for the South African small pelagics fishery was done to allow for a more multi-disciplinary and better ecosystems approach to the fishery (Nel, 2007).

The importance of distinguishing the different stocks of Sardinops sagax off southern Africa:

The notion of stock identification is of vital importance in fisheries management (Begg & Waldman, 1999; Begg *et al.,* 1999; Waldman, 2005). The definition of a stock is very broad and should evolve as the management requirements of a fishery change. It depends on the fishery and the characteristics of the species within that fishery, but generally, a stock is considered to be a homogenous population unit with similar characteristics, that is self-reproducing (Begg & Waldman, 1999; Begg *et al.,* 1999; Waldman, 2005; Lester & MacKenzie, 2009; Baldwin *et al.,* 2012).

Waldman (2005) defined a stock to be "a group of individuals of a particular species whose genetic characteristics, and usually life history characteristics, are more similar to each other than they are to those of other stocks." Phenotypic variation between stocks is also a well known phenomenon (Swain & Foote, 1999). Fish stocks can therefore be determined on the basis of variations in genotypic, phenotypic or life history characteristics (Begg *et al.,* 1999). Fish stocks can, however, show varying degrees of mixing with other stocks, which can complicate the identification of a discrete sub-unit of the population (Stephenson, 1999). Begg and Waldman (1999) suggest a holistic approach in identifying different fish stocks, where the use of multiple techniques can maximise the likelihood of correctly identifying fish stocks.

In order to effectively manage a fishery and implement successful stock rebuilding strategies, it is important to know the structure and characteristics of the stock being managed (Waldman, 2005). Disregarding the characteristics of a stock can lead to the ineffective management of a fishery and result in dramatic changes in the biological traits and productivity of a population (Begg & Waldman, 1999). Each stock will therefore require its own regulatory mechanisms to allow for the successful development of the fishery.

The South African sardine stock is presently managed as a single population; however, as briefly mentioned, there is a hypothesis that there are in fact two, perhaps even three phenotypically discrete stocks of sardine within South African waters (Coetzee *et al.,* 2008; van der Lingen, 2011; Department of Agriculture, Forestry and Fisheries*,* 2012). Putative western, southern and eastern stocks have been hypothesised, with the western and southern stocks occurring to the west and east of Cape Agulhas, respectively, while the eastern stock is thought to occur off the coast of KwaZulu Natal (Figure 1.2). The presence of more than one stock in South African waters will have profound implications on the management of the fishery within South Africa, where management plans may have to be amended in order to avoid the over utilisation of one stock. There are a range of studies that support the sardine multi-stock hypothesis (Miller *et al.,* 2006; Coetzee *et al.,* 2008; Wessels, 2009; van der Lingen *et al.,* 2010; van der Lingen, 2011; Reed *et al.,* 2012). These studies use a variety of different techniques and so support Begg and Waldman's (1999) concept of using a holistic approach to define discrete fish stocks, and will be discussed at a later stage.

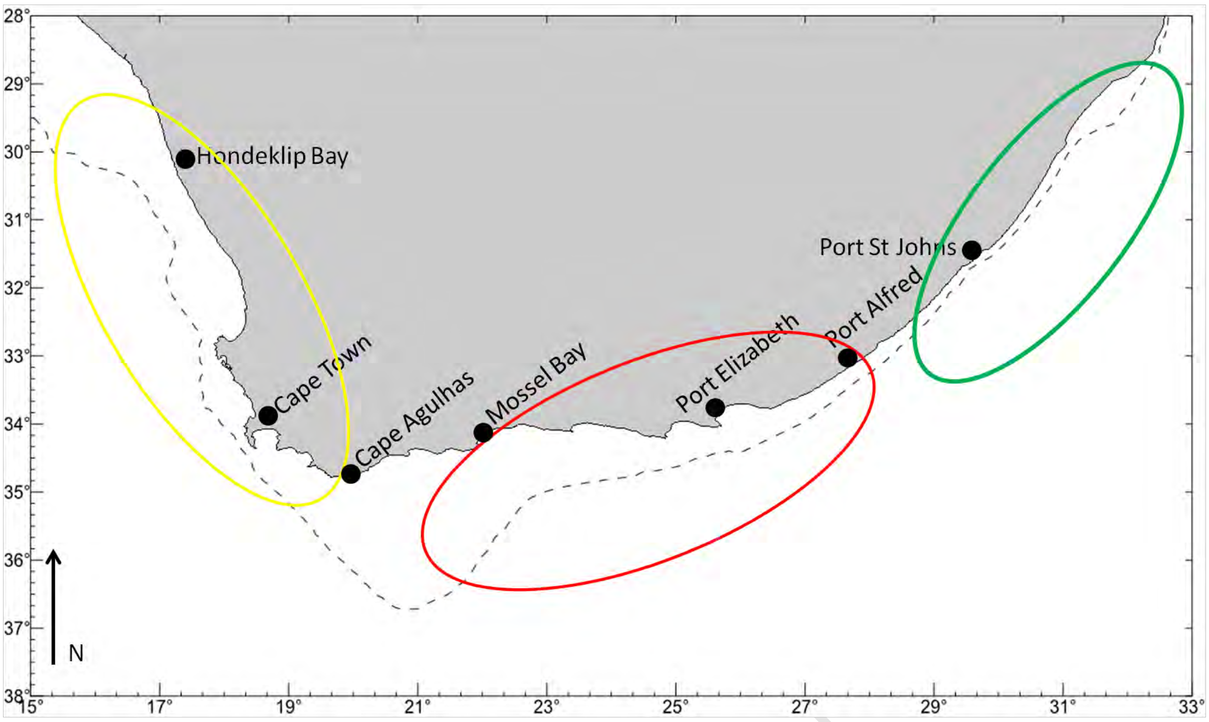

Figure 1.1: Map showing the location of the three putative stocks of *Sardinops sagax* thought to occur off the South African coast, where yellow indicates the putative western stock, red indicates the putative southern stock and green indicates the putative eastern stock.

In order to accommodate the findings of these various studies, advances in developing stock-specific assessment models for the putative western and southern subpopulations have been made (de Moor & Butterworth, 2012; de Moor & Butterworth, 2013). Preliminary projections from these models suggest that presently the abundance of the putative western stock is significantly below its long-term average, while the abundance of the putative southern stock is greater than its long-term average. Although this is the presently case, it is clear that the putative western stock is more productive than the putative southern stock, and the biomass of the southern stock depends heavily on recruitment from the west. This indicates mixing between the two putative stocks (de Moor & Butterworth, 2013). Further work and research is required, but the development of this model will assist in better assessment of the South African sardine resource, and once fully developed, may help in better management of the fishery.

Studies to support the multiple stock hypothesis of Sardinops sagax in southern African waters:

In southern African waters a variety of studies, using different techniques have supported the occurrence of multiple stocks of sardine. Coetzee *et al.* (2008) used results from the November acoustic surveys and identified a distinct separation in the distribution of *S. sagax* off the coast of South Africa at Cape Agulhas at low and medium biomass levels. This is shown in Figure 1.3. Sardine to the west of Cape Agulhas occupied the western Agulhas Bank at all biomass levels, and with increasing biomass, their distribution extended in a northerly direction, up the west coast, and in an easterly direction to the central Agulhas Bank. Sardine to the east of Cape Agulhas occupied the eastern Agulhas Bank, and with increasing biomass, their distribution extended in a westerly direction towards the central Agulhas Bank. It was only at a high biomass of *S. sagax* that it was found that there was an overlap between eastern and western parts of the population at the central Agulhas Bank.

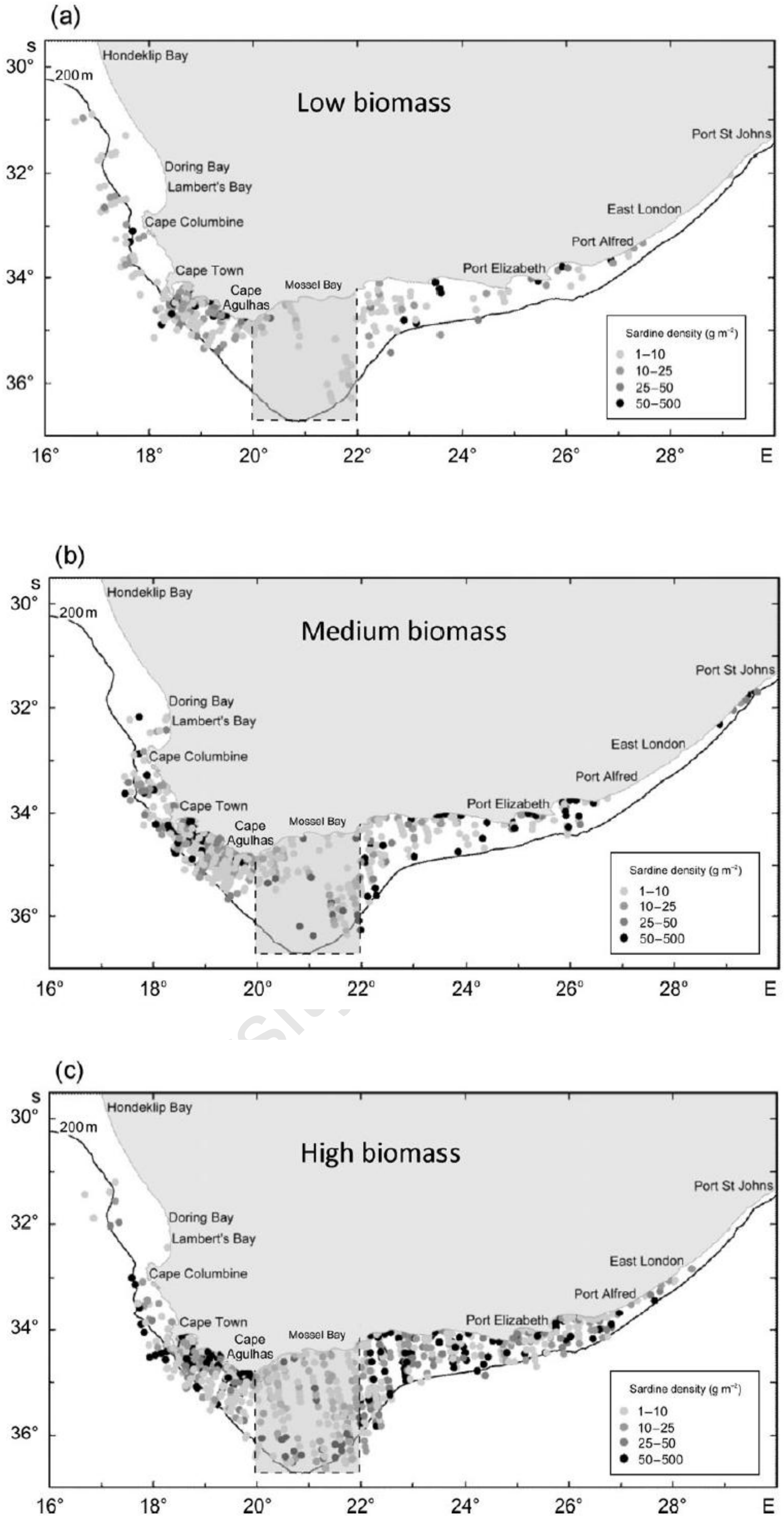

Figure 1.3: Composite density maps derived from November acoustic survey data showing sardine distributions during periods of (a) low, (b) medium and (c) high biomass. The block indicated by the dashed line indicates the transition zone between the west coast and south coast systems. The solid line represents the continental shelf. The separation in distribution between Cape Agulhas and Mossel Bay at low and medium biomass levels is obvious (from Coetzee *et al.,* 2008).

van der Lingen (2011) investigated sardine egg distributions collected from California Vertical Egg Tow (CalVET) samples collected during annual pelagic spawner biomass surveys conducted in spring, from 1986 to 2009, and used these data to plot a composite distribution map. The results, shown in Figure 1.4, show a clear separation in spawning areas at the Central Agulhas Bank. The western spawning area extends from Cape Agulhas, up the west coast to Hondeklip Bay, while the southern spawning grounds extend from the eastern part of the central Agulhas bank, along the south coast, to Port Alfred. Spawning during the sardine run occurs in a third spawning area off the east coast, but this is not shown in the figure.

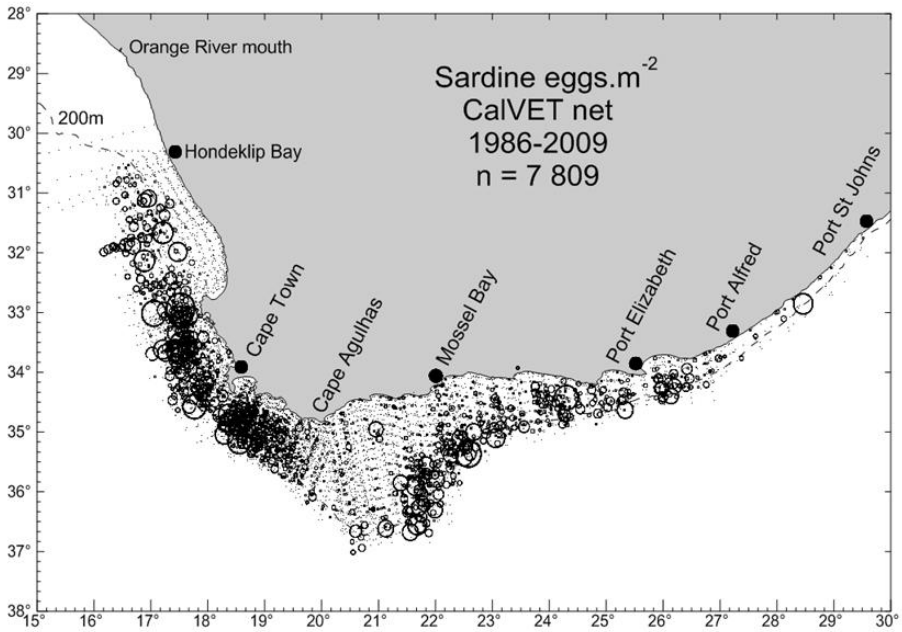

Figure 1.4: Composite sardine egg density map from CalVET net samples collected during spring from 1986-2009, showing the separation in egg density at the Central Agulhas Bank (from van der Lingen, 2011).

The consequences of the separation in the spawning areas of *S. sagax* off the South African coast, at Cape Agulhas, were investigated by Miller *et al.* (2006). Using different modelling techniques, it was found that different spawning areas led to differential retention and transport patterns in eggs and larvae, resulting in different life history strategies of sardines off the South African coast. The findings implied that the spawning of *S. sagax* off the South African coast is divided primarily into two different recruitment systems. The relative importance of each of these systems varies on an annual basis. For individuals that spawn on the western Agulhas Bank, recruits travel up the west coast, to the west coast nursery grounds, while for those individuals that spawn on the eastern Agulhas Bank, recruits mainly travel to the south coast nursery grounds. A third recruitment strategy was found where individuals are spawned on the eastern Agulhas Bank and recruited up the west coast, however, this transport does not occur extensively. Miller *et al.* (2006) also found that sardines had a possible third spawning area off the east coast of South Africa during the annual sardine run. East coast sardine could therefore present a further subpopulation off the coast of South Africa. This is supported by the findings of Freon *et al.* (2010).

Consistent differences in certain other characteristics have also been found between west, south and east coast sardine. These include differences in meristics such as vertebral count, where a significant difference was found in the number of vertebrae between west and south coast sardine, and east coast sardine (Wessels, 2009; van der Lingen *et al.,* 2010; van der Lingen, 2011). Significant differences in morphometrics such as body shape have also been found, where 16 of the 22 standard morphometric measurements were found to be significantly different between sardine from the three areas off the South African coast (Wessels, 2009; van der Lingen *et al.,* 2010; van der Lingen, 2011). Variability in sardine genetics was investigated by Hampton (2013), who used seven microsatellite loci and the mitochondrial marker ND2, and found some, albeit weak, differentiation between fish from the different regions. Additionally, differences in parasite prevalence rates and infection intensities have been found between sardine on the west and south coasts (van der Lingen, 2011; Reed *et al.,* 2012).

Studies to support the occurrence of multiple stocks of sardine in other areas of the world:

The concept of the occurrence of more than one stock within the overall distribution of *S. sagax* is not a foreign one, and may also occur in the Pacific where three phenotypically distinct subpopulations of *S. sagax* have been hypothesised (Felix-Uraga *et al.,* 2005; Martinez-Porchas *et al.,* 2009; Garcia-Rodriguez *et al.,* 2011). Felix-Uraga *et al.* (2005) used multivariate discriminant analysis to analyse the differences in otolith morphometrics between putative stocks of sardine in the Pacific. Their results supported the occurrence of three distinct sardine stocks within the study area. Martinez-Porchas *et al.* (2009) found that the distribution of each of these stocks was mainly related to temperature gradients. The "colder subpopulation" occurs off the coast of California in waters that range between 13 and 17°C, the "temperate subpopulation" occurs off the Baja California Peninsula in waters that range between 17 and 22°C, and the "warmer subpopulation" occurs off the Gulf of California in waters that are greater than 22°C.Given the extreme temperature gradients present in the waters off the coast of South Africa, different subpopulations of *S. sagax,* due to temperature gradients, may be a plausible explanation. Furthermore, Garcia-Rodriguez *et al.* (2011) used geometric morphometric body landmarks to analyse the morphology of sardines in the three areas of the Pacific, thought to consist of three distinct stocks. Clear morphometric differences were found between fish from the three groups, thus also supporting the occurrence of three discrete stocks of *S. sagax* off the coast of the Baja California Peninsula.

The occurrence of multiple sub-populations of sardine may also occur off the coast of Australia. Izzo *et al.* (2012) used two otolith based techniques to delineate the possible stock structure of the Australian sardine. Both otolith trace element and otolith shape analysis provided evidence for the presence of at least three major sardine sub-populations. These included South Australia, central Victoria and east coast Australia groups. Each of these subpopulations were shown to exhibit various levels of overlap with each other, but were all considered to be semi-independent, indicating the complexity of the population structure of sardine off the coast of Australia.

The use of parasites as biological tags to delineate Sardinops sagax stocks off southern Africa:

The basic principle:

The use of parasites as biological tags to differentiate different fish stocks is a method that has been widely employed, globally with a variety of different fish and parasite species, for many years (MacKenzie, 2002; Lester & MacKenzie, 2009). However, using parasites to distinguish stocks of *S. sagax* is the first time this method has been used in South African waters (Reed *et al.,* 2012). The basis for the use of parasites as biological tags in fish stocks is that a fish can only become infected by a parasite when it enters an endemic area where the parasite is present (MacKenzie & Abaunza, 1998). The endemic area is that geographical region where the transmission of parasites between hosts is enabled due to suitable environmental conditions. Marine parasite distribution patterns are driven particularly by temperature and salinity (Reed *et al.,* 2012). Because of this and due to the strong oceanographic gradients off the coast of South Africa, in terms of temperature and salinity (Hutchings *et al.,* 2009), the use of parasites as biological tags in South African waters is facilitated (Reed *et al.,* 2012). The endemic area of the parasite is smaller than that of the host distribution and so, if an infected fish is found outside of an endemic area, it can be assumed that the fish was once present within the endemic area, and depending on the life span of the particular parasite within the host, it can be determined the maximum time since the fish became infected (MacKenzie & Abaunza, 1998).

Advantages and disadvantages:

Parasites are naturally occurring within fish populations and so provide an array of advantages in their use as a tag to differentiate different stocks. They are well suited to the study of specifically smaller, more delicate species where the use of other methods such as artificial tags maybe very difficult or not possible. In larger species of fish, where the use of artificial tags is possible, there may still be doubts concerning abnormal behaviour of the fish, instigated by the tag. The use of parasites as tags in these species eliminates these uncertainties. Additionally, because they are naturally occurring, the use of parasites as tags in populations is inexpensive in comparison to other methods of tagging, and requires only the appropriate sampling of the concerned fish stock (MacKenzie & Abaunza, 1998). Having said this, there are also a variety of limitations in the use of parasites as biological tags that need to be considered. Firstly, it is usually advantageous to know the age of the host species in which the parasite is present; however aging techniques are not well established for many fish species and so the use of parasites as biological tags is limited. Also, many parasite species display complex biological and ecological characteristics. Thorough knowledge of the parasite species being used as the biological tag is therefore needed, but it cannot always be easily obtained. Additionally, there is much debate by taxonomists over the identification of certain parasite species, and so biological tag species need to be chosen carefully (MacKenzie & Abaunza, 1998).

Selection criteria of biological tags:

Because of their limitations, a variety of authors have established selection criteria for an ideal parasite to be used as a biological tag (Kabata, 1963; Sindermann, 1983; MacKenzie, 1983, 1987; Williams *et al.,* 1992, cited in MacKenzie & Abaunza, 1998). Generally, parasites very seldom fulfil all of the criteria, and so compromises do have to be made

(MacKenzie & Abaunza, 1998). Firstly, an ideal parasite should be easily detectable and identifiable, where minimum dissection of the host is necessary; otherwise time may become a limiting factor. The chosen parasite should not significantly affect the behaviour of the host, nor should it be a serious pathogen that may cause selective mortality in hosts. The parasite should also have significantly different levels of infection of the host within different parts of the study area. This allows for the data to be analysed in terms of prevalence, intensity and abundance of infection. Additionally, the parasite should also have a relatively long life span in the host, in order to allow for sufficient time to analyse the infection within a population. For stock identification specifically, parasites need to have a life span of at least 1 year within a host in order to allow for adequate identification. Parasites with a single-host life cycle are easiest to use as biological tags. Parasites with more than one host are more complex to use because more information is required about the transmission of the parasite between the different hosts. However, if this information is attainable, the parasite may be just as effective as a biological tag. Finally, and perhaps of most importance for this study, is that the infection of the host by the chosen parasite should be stable with time, and so ideally should not show any seasonal or interannual variation in infection.

Previous studies on the use of parasites as biological tags in small pelagic species:

There are a vast number of studies on the use of parasites as biological tags to differentiate different stocks and identify movement patterns of a variety of small pelagic species. Many of these studies have been done on carangid species of the genus *Trachurus,* or mackerel species (MacKenzie, 2002). A study conducted on the Pacific coast of South America used a combination of parasites as biological tags and morphometrics to distinguish migration patterns of the Pacific jack mackerel *Trachurus symmetricus murphyi*. The study showed that, in south central Chile, the Pacific jack mackerel population migrated coastwards in winter, however in the north of Chile, the population did not display this pattern (George-Nascimento

& Arancibia, 1992, cited in MacKenzie, 2002). A variety of parasites were found to be of use in identifying this. A further study identified two isopod parasites in particular, from the genus *Ceratothoa,* that showed significant reproductive differences between Pacific jack mackerel caught off south central Chile and those caught off the coast of northern Chile, thus suggesting that there are two separate stocks of the host population present in these two regions (Aldana *et al.,* 1995, cited in MacKenzie, 2002).

Pozdnyakov and Vasilenko (1994) conducted a successful parasite study to identify separate stocks of the Japanese mackerel *Scomber japonicus* in the northwest Pacific. Helminth parasite infections in the Japanese mackerel in the region were studied over a period of nine years where it was found that the host populations differed significantly in their composition of parasite fauna between the coastal and oceanic regions. This indicated the possible presence of two separate stocks of Japanese mackerel- one northern stock and one southern stock. The northern stock were primarily infected by parasites that required shallower, coastal areas to complete their life cycle, while the southern stock were primarily infected by parasites that were characteristic of the open ocean.

Another study on parasites in small pelagics was conducted by Moser and Hsieh (1992), who looked at parasite fauna in the Pacific herring *Clupea harengus pallasi* in California, as possible biological tags in order to distinguish the existence of different stocks. Past evidence for the presence of more than one stock of Pacific herring off the coast of California was inconclusive (Worthy, pers. comm., Scott, pers. comm., Spratt, 1981, cited in Moser & Hsieh, 1992) and therefore, the use of parasites as biological tags was suggested. Herring were sampled from three sites over a period of nine years, and it was found that the cestode *Lacistorhynchus dollfusi,* various nematodes including *Anisakis simplex* larvae, and the adult digenean *Parahemiurus merus* were of use as tags, and were able to distinguish two spawning populations that remained separate outside of the spawning season between San

Francisco and Tomales bays. This implied the presence of two stocks of Pacific herring off the Californian coast.

Timi (2003) used parasites as biological tags and identified four stocks of the Argentinean anchovy *Engraulis anchoita* in the south-west Atlantic. Here, latitudinal difference seemed to play a significant role in the composition of the parasite populations, where the changes in environmental conditions, such as temperature and salinity, with latitude, may have had an influence on the endemic areas inhabited by the parasites studied (Timi, 2003; Timi, 2007). Additionally, statistical analysis of the parasite samples obtained indicated that there was a clear seasonal impact where, in general, there was greater prevalence of parasite species during spring in comparison to autumn which was characterised by low prevalence of most parasite species (Timi, 2003). Among the parasites used as tags to distinguish the different stocks of anchovy, "tetracotyle" type metacercariae from the genus *Cardiocephaloides,* that were found in the eye, were used and were particularly prevalent in anchovy in southern waters, enabling the identification of the southern stocks (Timi, 2003; Reed *et al.,* 2012).

Previous studies on the use of parasites as biological tags in Sardinops sagax in particular:

Although some research has been conducted on sardine parasites in general, Reed *et al*. (2012) conducted the first study in South African waters looking at parasites as possible biological tags to distinguish different stocks of *S. sagax.* A preliminary survey of the parasite fauna of *S. sagax* was completed, where seven parasite species were identified. It was found that the digenean metacercariae of the "tetracotyle" type species from the genus *Cardiocephaloides,* was most prevalent, where infections of the humours of the eyes were found in 46% of fish examined (Reed *et al.,* 2012). A photograph of this parasite *in situ* is shown in Figure 1.5. This genus of this parasite was also used in stock identification of Argentinean anchovy in the south-west Atlantic (Timi, 2003). Spatial variation in prevalence of infection was also found with the “tetracotyle” type species, where samples obtained from the west coast were found to have higher values of prevalence in comparison to samples from the south coast of South Africa (Reed *et al.,* 2012). Because of this variation in the distribution of the “tetracotyle” type species, and because these parasites seem to fulfil majority of the requirements of a successful biological tag, it was concluded that the “tetracotyle” digenean has the most potential to be used as a biological tag to distinguish the presence of difference stocks of *S. sagax* off the coast of South Africa. Most importantly the “tetracotyle” parasite was found to be easily identifiable in the eyes of the sardine, the parasite also seemed to have different levels of infection in sardines within South African waters, because it is found internally it presumably has a relatively long life span in the sardine, and although the exact details of the life cycle are not known, basic information on the hosts involved is attainable. Further research, with greater sample sizes, on the geographical and temporal distribution of the parasite, as well as its occurrence in different age and size classes of sardine, however, needs to be conducted.

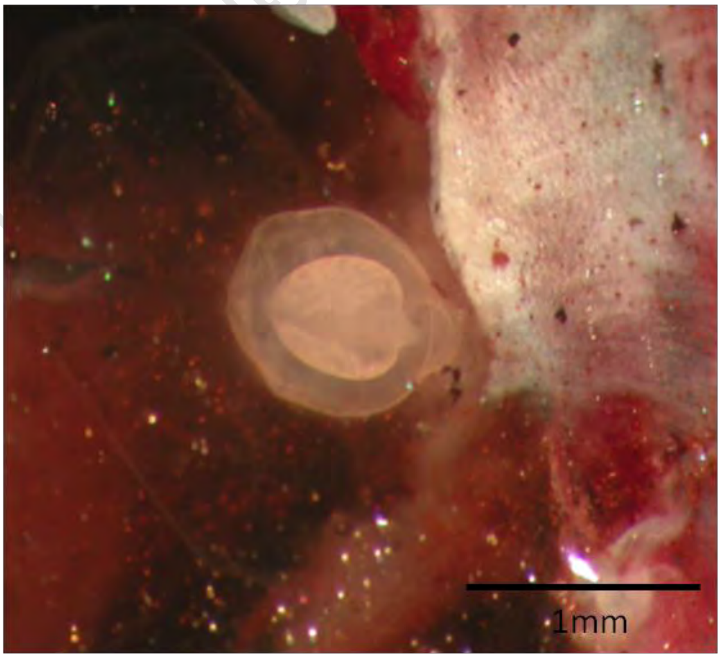

Figure 1.5: “Tetracotyle” type metacercaria *in situ* (from Reed *et al.,* 2012).

"Tetracotyle" type metacercariae:

Taxonomy:

Following the survey of the parasite fauna present in *S. sagax* off the coast of South Africa, it was found that the "tetracotyle" type species were of most use as a possible biological tag because this parasite fulfilled majority of the selection criteria (Reed *et al.,* 2012). "Tetracotyle" type species are digeneans and fall into the class Trematoda, or the Platyhelminthes (Cribb, 2005). The digenea are an extremely large and diverse group of endoparasitic flukes and because of this, classification of them is very difficult (Cribb, 2005). "Tetracotyle" type species are however, characteristic of the metacercariae in the superfamily Diplostomoidea and the family Strigeidae (Niewiadomska, 2002b). The metacercariae in closely related genera within the family Strigeidae are all relatively similar, and so generic names have been established in order to distinguish them- the "tetracotyle" type forms one of these groups (Niewiadomska, 2002b). As in the Argentinean anchovy, the "tetracotyle" type metacercariae may belong to the genus *Cardiocephaloides,* where the species *Cardiocephaloides physalis* may be of most interest as a biological tag in the South African sardine (Timi *et al.*, 1999; Reed *et al.,* 2012).

Morphology:

Sexual adults from the family Strigeidae are typical of the general digenean morphology, where a cup-shaped forebody is present with a holdfast organ or sucker in the form of a ventral and dorsal lobe (Niewiadomska, 2002a). An oral sucker is usually present that opens directly into the oesophagus and gut, and the reproductive organs occur primarily within the hindbody (Niewiadomska, 2002a).

The morphology of the metacercariae from the Strigeidae family are well known, allowing classification of them into different groups, and making them good candidates for biological tags. This classification is primarily based on the structure of the reserve bladder (Niewiadomska, 2002b). The group of "tetracotyle" type metacercariae have a cup shaped forebody, and a smaller hindbody. This is encapsulated in a cyst wall. Pseudosuckers are present, and the structure of the reserve bladder is much more complicated compared to the other groups of Strigeidae metacercariae. The reserve bladder within "tetracotyle" forms a net that fills the entire body, and the excretory bodies are free within the canals of the reserve bladder (Niewiadomska, 2002b). A diagram and a photograph of two "tetracotyle" type metacercariae are shown in Figures 1.6 and 1.7.

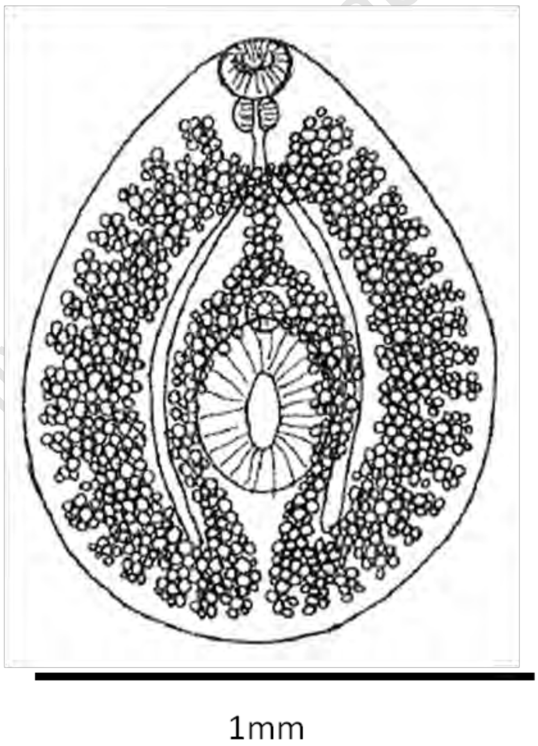

Figure 1.6: Diagram of the "tetracotyle" type metacercaria (from Niewiadomska, 2002b).

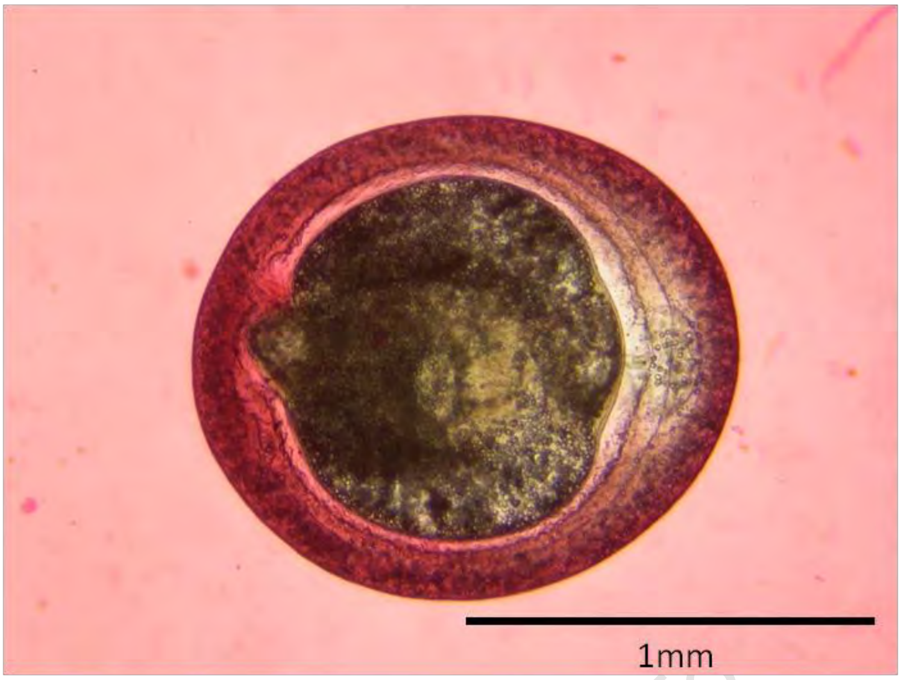

Figure 1.7: Photograph showing a fresh “tetracotyle” type metacercaria stained with Mexican Red textile stain (from Reed *et al.,* 2012).

Life cycle:

The digenean life cycle is usually relatively complex where more than one host is required, both free-living and parasitic stages are present and sexual and asexual reproduction is always present. Majority of the group can affect marine animals in some way or another. Majority of digeneans initially infect a mollusc first intermediate host, are transmitted to a second intermediate host, and then finally are transmitted to infect a vertebrate definitive host. In particular, species from the family Strigeidae, have a bird or mammal definitive host (Niewiadomska, 2002a).

Adults sexually reproduce to form eggs that pass into the environment. These eggs hatch to form a miracidium which is a motile, non-feeding and relatively short-lived larva. The miracidium swims and shortly afterwards, penetrates the first intermediate host, which is a mollusc, specifically a gastropod. Digeneans generally have a large impact on the health of this first intermediate host. Once the miracidium has penetrated the molluscan host, it forms a mother sporocyst, which produces the second generation of either daughter sporocysts or rediae within the host, asexually. The difference between the daughter sporocysts and the rediae lies primarily in the way in which they feed. The daughter sporocysts are similar to the mother sporocysts and lack any feeding structures or gonads, so food is absorbed directly through the tegument. Rediae, in contrast, have a mouth, pharynx and short saccular gut. This generation dominates the digestive system and gonads of the molluscan host and reduces their reproductive output significantly or even entirely. The molluscan host will generally remain infected with the digenean parasite for life. The daughter sporocysts or rediae both reproduce asexually, in a similar way to the mother, to produce cercariae. The cercariae are free-swimming, usually with a tail, and emerge from the molluscan host, most often through the gill chambers. The cercariae then enter the second intermediate host through two different ways, either penetration of the host directly or being eaten by the host. Once they have entered the second intermediate host, the cercariae become metacercariae and are embedded within certain tissues and no longer reproduce, thus are relatively benign. At this stage, the cercariae fulfil the criterion of a good biological tag as they do not seem to be serious pathogens to the host. The digenean parasite then finally enters the definitive vertebrate host, most commonly through ingestion, when the definitive host feeds on the intermediate host as prey. Within the definitive host, overall, digeneans do not seem to be serious pathogens and so do not cause significant harm (Cribb, 2005).

The "tetracotyle" type metacercariae from the Strigeidae family infect a variety of different hosts, including fish (Niewiadomska, 2002b). *Sardinops sagax* therefore acts as the second intermediate host in the life cycle of this parasite, where it infects the humours of the eyes. Previous studies, some from as early as 1832, have shown that for many Strigeidae species, the eye of the fish plays an important role in the survival of the larval stage, where it is often the preferred habitat for the metacercariae (von Nordmann, 1832; Steenstrup, 1842; Szidat, 1924, cited in La Rue *et al.,* 1926). In this second intermediate host, the presence of the metacercariae does not seem to cause direct damage to the fish where the parasites do not seem to have any effect on the tissues of the eye (La Rue *et al.,* 1926). However, if found in very high abundances, some authors have recorded an affect of various different Strigeidae species on the vision of the fish (von Mordmann, 1832; Matare, 1910; Szidat, 1924 cited in La Rue *et al.,* 1926).

Details of the other species involved in the "tetracotyle" type parasite life cycle are still unknown, however, it is thought that the first intermediate host of the "tetracotyle" type metacercaria is a type of gastropod, and the definitive vertebrate host is a fish-eating marine bird (Niewiadomska, 2002a; Reed *et al.,* 2012). It has been suggested that the "tetracotyle" type metacercariae found in *S. sagax* may be from the species *Cardiocephaloides physalis* (Timi *et al.*, 1999; Reed *et al.,* 2012). *Cardiocephaloides physalis* is a parasite found in penguins, and has been found in jackass penguins, *Spheniscus demersus,* in South African waters (Randall & Bray, 1983; Horne *et al.,* 2011; Reed *et al.,* 2012). *Spheniscus demersus* may therefore act as the definitive host of the "tetracotyle" type species found in *S. sagax* in South African waters. Further research regarding this is currently being conducted.

Seasonality:

There is very little information available on whether the "tetracotyle" type metacercariae display any seasonality in their prevalence, abundance and infection intensity within hosts. However, Timi (2003) showed that prevalence and abundance of parasite species in general, in Argentine anchovy was significantly lower in fish caught in autumn, compared to fish caught in spring. This pattern was no different for the *Cardiocephaloides* species, of which the "tetracotyle" type metacercaria is thought to be a part (Timi, 2003). However, this result may be an artefact of the sampling method used in this study, whereby, the number of fish caught and examined for parasites differed significantly between autumn and spring. The sample number in autumn was 44 fish, while in spring the sample number was 627 fish. Other factors such as the geographic origin of the samples were also found to play a role in the apparent seasonality found in this study.

The use of "tetracotyle" type metacercariae, found in S. sagax, as a possible biological tag:

Due to its characteristics and the findings from the previous study conducted by Reed *et al.* (2012), the "tetracotyle" type metacercaria found in *S. sagax* off the South African coast does therefore seem to fulfil majority of the selection criteria for a successful biological tag (Kabata, 1963; Sindermann, 1983; MacKenzie, 1983, 1987; Williams *et al.,* 1992, cited in MacKenzie & Abaunza, 1998). The life cycle of the "tetracotyle" parasite may be slightly more complex than what is desirable, however parasites that are used as biological tags very seldom fulfil all of the selection criteria (MacKenzie & Abaunza, 1998). Furthermore, progress is being made regarding the details of the life cycle of this species and so with increased knowledge, this species may be just as effective in distinguishing stocks of *S. sagax*. The possible seasonality of the occurrence of the "tetracotyle" type metacercaria may, however, pose a problem to its success as a biological tag off the coast of South Africa. Although Timi (2003) showed a possible seasonal pattern in this species, further research, with increased sample sizes, does need to be conducted in order to support this and determine its usefulness as a potential biological tag.

Aims and hypotheses:

Overall, this study aimed to apply the method and principle of using parasites as biological tags in order to test the hypothesis of multiple stocks of sardine off the coast of South Africa.

Firstly the study aimed at confirming the hypothesis that prevalence, infection intensity and abundance of "tetracotyle" type metacercariae in South African sardine show spatial variation, as observed by Reed *et al.* (2012). Given previous results, it was hypothesised that there would be a significant spatial variation in these indices, which were anticipated to be higher in fish from the putative western stock than in those from the putative southern stock. The null hypothesis of no spatial difference in parasite prevalence, infection intensity and abundance was therefore used, and rejection of this hypothesis would support the existence of these two discrete stocks.

Secondly, and since seasonality or interannual variability in infection of *S. sagax* by this parasite may confound the conclusions drawn from the results of spatial variation in infection of the parasite in *S. sagax*, the study aimed at testing whether these indices show any temporal variation. Should such be encountered it would need to be accounted for when considering spatial variability in parasite indices. Hence the null hypothesis of no temporal variation, either seasonal or interannual, in prevalence, infection intensity and abundance of the "tetracotyle" parasite was used.

CHAPTER 2: Materials and Methods

Sampling:

Sardine samples were obtained from commercial catches that were taken at different localities and landed at four different harbours around the South African coast, over a period of approximately two years, from the beginning of 2011 to the end of 2012. Locality is defined to be the "geographic locale of the external environment where the parasite is found" (Bush *et al.,* 1997). Attempts were made to collect one sample of 25 sardines per month from each harbour, but spatio-temporal variation in sardine availability to the fishery precluded consistent monthly coverage of all harbours. The occurrence of "tetracotyle" type metacercariae was compared for *S. sagax* caught from either side of Cape Agulhas, where it is assumed that the separation of the putative stocks of sardine off the South African coast occurs (Miller *et al.,* 2006; Coetzee *et al.,* 2008; Wessels, 2009; van der Lingen, 2011; Reed *et al.,* 2012). Sardine caught at localities to the west of Cape Agulhas were assumed to be a part of the putative western stock, and were landed at either Gans Bay or St. Helena Bay, while fish caught at localities to the east of Cape Agulhas were assumed to be a part of the putative southern stock, and were landed at either Mossel Bay or Port Elizabeth. The locations of the samples analysed were plotted on a map using the Surfer® 10 software.

Processing:

Once collected, the samples were either frozen or preserved in ethanol, and their date and catch locality recorded. Where possible, each of the 25 fish from each month from each putative stock were sexed and measured in terms of caudal length, to the closest millimetre. The left and right eye were removed and examined under the microscope at 1x magnification. During the dissections, no other parasites were found in the eyes of the sardine specimens, and so it was assumed that all parasites observed were "tetracotyle" type metacercariae.

The “tetracotyle” type metacercariae in each eye were counted, systematically, and recorded, and the prevalence of infection, mean infection intensity and mean parasite abundance were calculated for each sample.

Definitions:

Bush *et al.* (1997) explicitly defines the terms infection prevalence, infection intensity and parasite abundance as quantitative measurements of parasite populations. Prevalence is defined as “the number of hosts infected with one or more individuals of a particular parasite species, divided by the number of hosts examined for that parasite species” (Bush *et al.,* 1997), and is therefore commonly expressed as a percentage. It is an important measurement in this study because it allows for the comparison, between putative stocks, of the percentage of fish infected by the “tetracotyle” parasite. Infection intensity is defined as “the number of individuals of a particular parasite species in a single infected host” (Bush *et al.,* 1997). Infection intensity therefore does not include those hosts that are not infected with the parasite, and so zero values are discounted. Infection intensity is an important measurement in this study because it allows for the comparison between putative stocks of the level of infection of the infected fish. Parasite abundance is defined as “the number of individuals of a particular parasite species in a single host, regardless of whether or not the host is infected” (Bush *et al.,* 1997), and so, contrary to the infection intensity, does include zero values that originate from uninfected hosts. The distinction between infection intensity and parasite abundance is important for the comparison of hosts from different hypothetical stocks as a stock with many zero values in terms of parasite abundance may be of significant importance (Bush *et al.,* 1997).

Data exploration:

In order to observe and statistically analyse the data, the number of parasites in each eye of each fish were combined, and so no distinction was made between parasites from the left and right eye. Prevalence and the means (±SE) for infection intensity and parasite abundance were plotted to show both spatial and seasonal variation between the putative western and southern stocks of *S. sagax* in the southern Benguela. Since there was not equal coverage of samples from the western and southern regions, months were rather converted to days, where 1 January 2011 was considered to be day one. Furthermore, days were grouped into four seasons, where December to February was considered to be summer, March to May was considered to be autumn, June to August was considered to be winter and September to November was considered to be spring.

In order to examine spatial variation in prevalence, infection intensity and parasite abundance, all of the values obtained for each putative stock were combined, and no distinction between months and years was made. Seasonal variation was examined by separating prevalence, infection intensity or abundance values for each season and so distinction was made between seasons. To account for a year effect that may be present in the seasonal variation in "tetracotyle" type metacercaria prevalence, infection intensity and abundance, the data for each year was normalised by expressing it as a proportion of the maximum value for that year. This data was also plotted.

Because size effects on parasite loads have been observed in other species (Timi & Poulin, 2003), it became clear that the size of the sardine in this study may have an influence on the number of parasites present. It was therefore hypothesised that the "tetracotyle" parasite may have a cumulative effect on *S. sagax* specimens, whereby as fish get older and grow larger, they become more infected. To test for this, caudal length was plotted against parasite infection intensity and parasite abundance. The relationship between the two variables was tested using a linear regression.

The relationships for infection intensity and abundance were then compared between the putative western and southern stocks using a Student's t-test to compare two slopes, where:

$$t = \frac{b_1 - b_2}{s_{b_{1}-b_{2}}}.$$

b_1 and b_2 are the slope values for the western and southern stock, while s_{b_1} and s_{b_2} are the standard error values for each respective slope (Zar, 1996). All data exploration was conducted using the computer packages Microsoft Excel 2007 and STATISTICA 11.

Statistical analyses:

The calculation of the prevalence of infection, mean infection intensity and mean parasite abundance, of the "tetracotyle" type metacercariae, allowed for the statistical comparison of these three measurements, between the putative western and southern stock in each month. Since year and length may have a significant influence on the prevalence, infection intensity and abundance of "tetracotyle" type metacercariae in *S. sagax* individuals from the putative western and southern stocks off the coast of South Africa, these variables were also taken into account in the statistical analyses.

The normality of the infection intensity and abundance of the "tetracotyle" parasite in sardine from both the putative western and southern stocks was tested using a frequency distribution. The normality of the prevalence data was not tested because prevalence had a binomial distribution, where there were only two categories- infected and uninfected.

Both the infection intensity and abundance data was found to be not normally distributed, and so the data did not fulfil the assumptions of a parametric General Linear Model. To avoid transforming the data and to increase the strength of the analyses, three Generalized Linear Models were therefore used to analyse the prevalence, infection intensity and abundance data.

In each model, stock, season and year were set as categorical variables while length was set as a continuous variable. Prevalence, infection intensity or abundance was treated as the dependent variable (Table 2.1). Each model included a two way interaction between season and stock. Year was not considered as an interactive term.

The full model was therefore of the form:

$$G(y) = stock + season + year + length + stock * season + error$$

Where y is the dependent variable- either prevalence, infection intensity or parasite abundance of "tetracotyle" type metacercariae found in *S. sagax* specimens.

Table 2.1: Predictor variables used in the Generalized Linear Models for the analysis of "tetracotyle" type metacercariae abundance, infection intensity and prevalence in *S. sagax* off the coast of South Africa.

Predictor	Type	No. of levels	Description
Stock	Categorical	2	Putative stock to which sardine specimens belong, based on the collection locality: western and southern
Season	Categorical	4	The four periods that the samples were divided into based on when they were collected- autumn, winter, spring and summer.
Year	Categorical	2	The two years over which all of the samples were collected- 2011 and 2012.
Length	Continuous	1	Caudal length, to the nearest millimetre of each of the *S. sagax.* specimens examined

The prevalence of parasites was treated as a binary response variable (1 = presence; 0 = absence) and therefore modelled using a binomial distribution, which was realised through the logit link function (Podolska & Horbowy, 2003). This was of the form:

$$\text{logit}\left(\frac{p}{1-p}\right) = x^T\beta$$

where p is the probability of at least one parasite being present, and x^T denotes the vector of predictor variables and β is the vector of the model coefficients (O'Neill & Faddy, 2003).

Since infection intensity and abundance were derived from parasite counts, the Poisson and negative binomial distributions were considered as two alternative error models that are suitable for modelling count data (Zuur *et al.,* 2009). Initially, a Poisson model was fitted to the data to identify potential over-dispersion, which is evident in cases where the variance is considerably greater than the mean. Where there was no evidence for over-dispersion, the response was assumed to follow a Poisson distribution, otherwise, the final model was fitted by assuming a negative binomial distribution, which is more suited to count data with high variance (Podolska & Horbowy, 2003; Penston *et al.,* 2008; Zuur *et al.,* 2009). Both the Poisson distribution and the negative binomial were fitted using the log-link function of the form:

$$\log(\mu) = x^T\beta$$

where μ is the mean and again $x^T\beta$ is a linear function of the model coefficients and the corresponding covariates stock, season, year, length and the interaction between stock and season (O'Neill & Faddy, 2003).

Infection intensity represents a special case of zero-truncated count data, as the response only comprises non-zero counts of parasites per fish. Therefore, the expected infection intensity needed to be corrected for the fact that the data set contained no zeros, whereby omitting this correction would result in the relative model under stating the predicted infection intensity (Welsh *et al.,* 1996; O'Neill & Faddy, 2003). The expected value for infection intensity, $E(y)$, for the zero-truncated Poisson distribution was given by:

$$E(y) = \frac{\mu}{1 - exp^{(-\mu)}}$$

and $E(y)$ for the zero-truncated negative binomial distribution was given by:

$$E(y) = \frac{\mu}{1 - (1 + \delta\mu)^{-\delta^{-1}}}$$

where μ is the mean infection intensity predicted by the untruncated count model and δ is the estimated dispersion parameter for the negative binomial distribution (Welsh *et al.,* 1996; O'Neill & Faddy, 2003).

The most parsimonious model for each analysis was selected by using the Akaike's Information Criterion (AIC) (Zuur *et al.,* 2009). This allowed for the selection of the optimal combination of predictor variables, and their interactions, in the final model. Additionally, the continuous variable, length, was analysed in both its untransformed and natural logarithm transformed states. Using the AIC, it was found that log transformed length provided the best fit for each model, and so length was logged for each analysis.

The significance of the factor levels was tested using sequential χ^2 tests, where the change in deviance as a result of adding an additional factor is approximately χ^2 distributed. Therefore, the χ^2 statistic can be used to calculate the significance of adding a factor to a model, based on the degrees of freedom (Podolska & Horbowy, 2003; Winker *et al.,* 2013). The derived *p*-values were used to determine the significance level of the variation in the data that is accounted for by each predictor variable. The total deviance explained by the final model can be expressed in the form of the pseudo coefficient of variation, which is calculated from the ratio of the total deviance explained by the best fitting model to the deviance of the null model, such that:

$$R^2 = 1 - \frac{Residual\ deviance}{Null\ deviance}$$

This statistic therefore provided a goodness-of-fit measure for the relevant model.

Finally, predicted values from each of the analyses were standardised by fixing all covariates, other than the variables of interest, to standardised values in order to plot the effects of individual predictor variables on the prevalence of infection, infection intensity and parasite abundance.

All statistical analyses were conducted using the computer packages Microsoft Excel 2007 and MASS, a default package in the statistical programme, R 2.15.2 (R Development Core Team, 2011).

CHAPTER 3: Results

Overall, a total of 733 sardine were dissected and examined for "tetracotyle" type metacercariae. Of these, 408 were from the putative western stock, collected from 17 monthly samples, while the remaining 325 were from the putative southern stock, collected from 13 monthly samples (Table 3.1). The localities of all samples analysed is shown in Figure 3.1, and locality by season and year for the putative western and southern stock is shown in Figures 3.2 and 3.3.

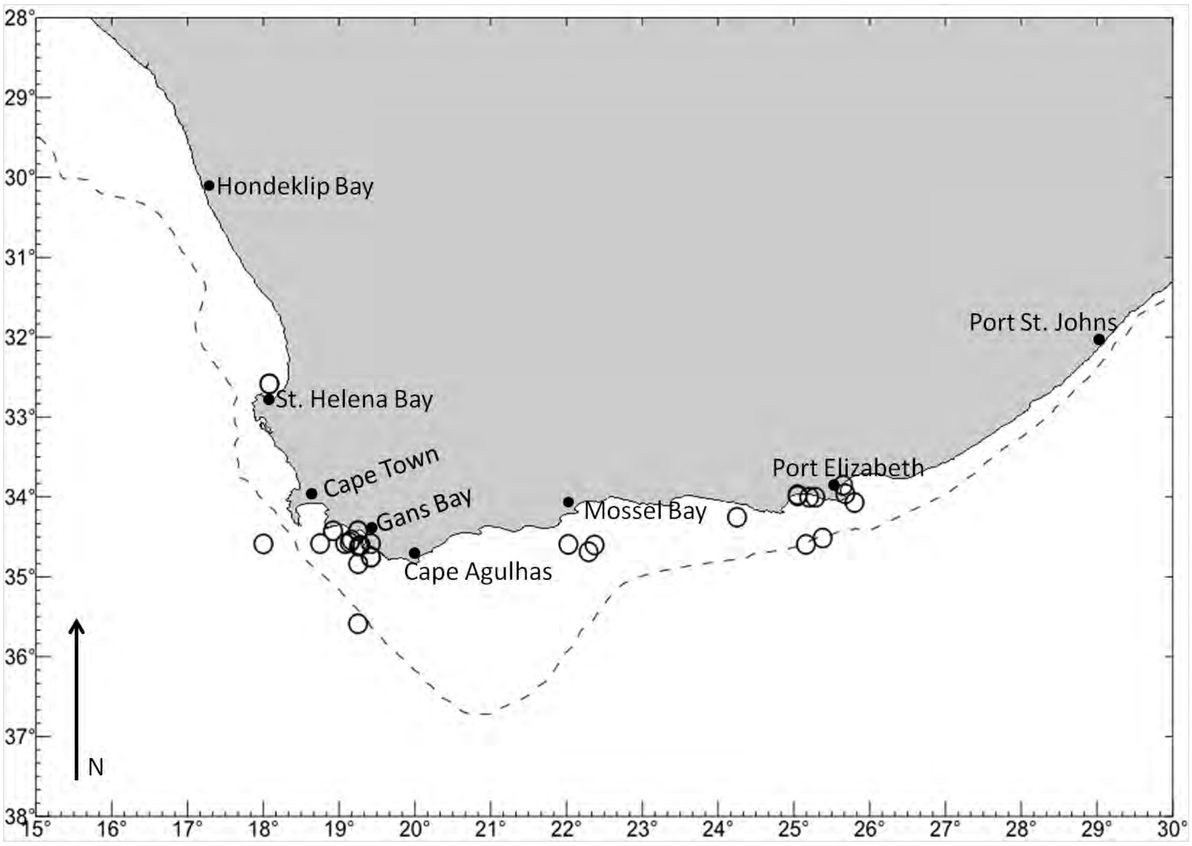

Figure 3.1: Map of the South African coast, showing the locality of all samples of *Sardinops sagax* collected and used for this study. The dashed line represents the continental shelf.

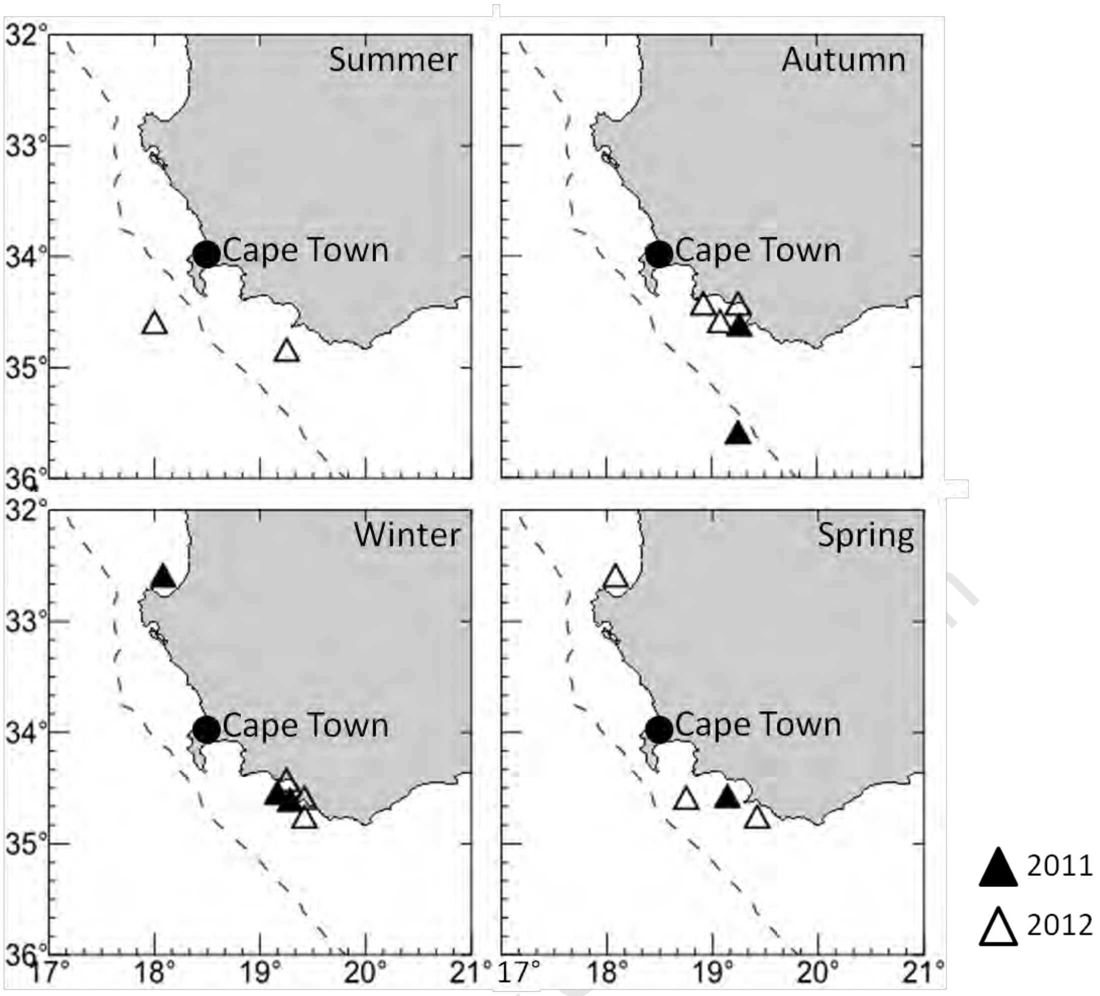

Figure 3.2: Map of the South African coast off Cape Town, showing the samples of *Sardinops sagax* collected to the west of Cape Agulhas, in each season, for 2011 and 2012. The dashed line represents the continental shelf.

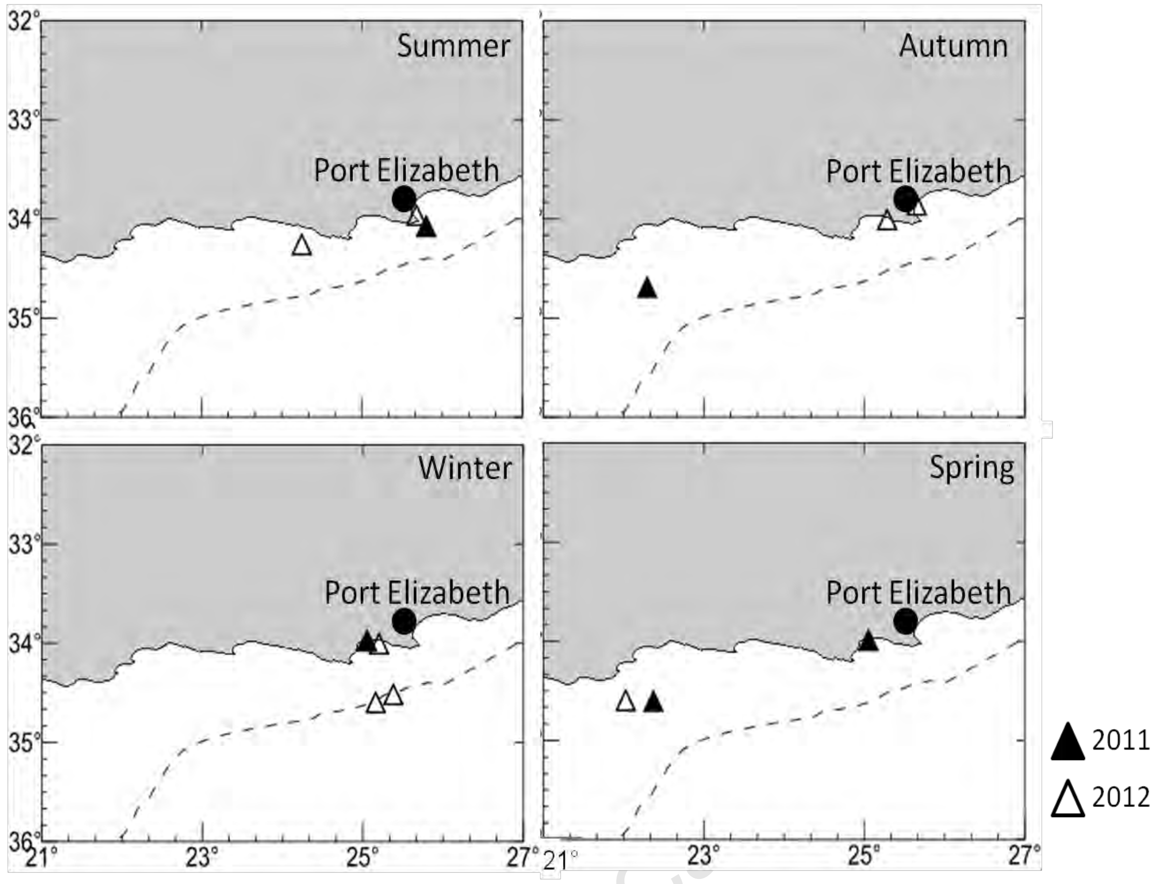

Figure 3.3: Map of the South African coast off Port Elizabeth, showing the samples of *Sardinops sagax* collected to the south of Cape Agulhas, in each season, for 2011 and 2012. The dashed line represents the continental shelf.

"Tetracotyle" type metacercariae were found in *S. sagax* from both the putative western and southern stocks, in samples that were frozen and samples that were preserved in ethanol (Figures 3.4 and 3.5).

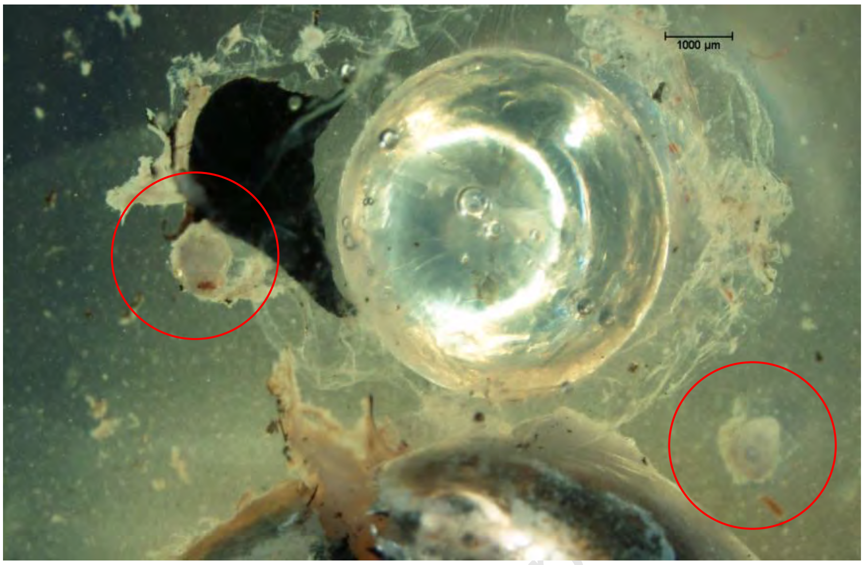

Figure 3.4: Picture showing the dissected eye of a frozen *Sardinops sagax* specimen, where red circles indicate the presence of "tetracotyle" type metacercariae (1x magnification).

Figure 3.5: Picture showing the dissected eye of a *Sardinops sagax* specimen that has been preserved in ethanol, where red circle indicates the presence of "tetracotyle" type metacercariae (1x magnification).

Table 3.1: Raw data showing individual samples and overall variation in mean (±SE) prevalence, infection intensity and abundance of “tetracotyle” type metacercariae in *Sardinops sagax* from the putative western and southern stocks off the coast of South Africa. Information on the date, day number (from 01/01/2011), season and locality of each sample is shown.

Stock	**Date**	**Day #**	**Season**	**Latitude**	**Longitude**	**n**	**Prevalence (%)**	**Mean(±SE) infection intensity**	**Mean(±SE) abundance**	**Preservation**
Western	02/03/2011	61	Autumn	-34.61	19.26	25	52	3.08(±0.97)	1.6(±0.59)	Frozen
Western	09/05/2011	129	Autumn	-35.58	19.25	25	32	2.25(±0.62)	0.72(±0.29)	Frozen
Western	30/06/2011	181	Winter	-32.58	18.08	16	68.75	3.81(±0.95)	2.63(±0.79)	Frozen
Western	18/07/2011	199	Winter	-34.60	19.28	24	83.33	3.8(±0.75)	3.17(±0.69)	Frozen
Western	02/08/2011	214	Winter	-34.53	19.16	22	95.45	3.19(±0.43)	3.05(±0.43)	Frozen
Western	06/09/2011	249	Spring	-34.57	19.14	21	90.48	3.47(±0.42)	3.14(±0.44)	Frozen
Western	25/01/2012	390	Summer	-34.83	19.25	25	92	2.61(±0.29)	2.4(±0.31)	Ethanol
Western	15/02/2012	411	Summer	-34.58	18.00	25	88	3.91(±0.58)	3.44(±0.57)	Ethanol
Western	08/03/2012	433	Autumn	-34.42	19.25	25	84	5.57(±1.05)	4.68(±0.91)	Ethanol
Western	04/04/2012	460	Autumn	-34.58	19.08	25	88	5.36(±1.49)	4.72(±1.35)	Ethanol
Western	10/05/2012	496	Autumn	-34.42	18.92	25	96	6.71(±0.86)	6.44(±0.86)	Ethanol
Western	11/06/2012	528	Winter	-34.75	19.42	25	88	14.55(±3.65)	12.8(±3.34)	Ethanol
Western	18/07/2012	565	Winter	-34.58	19.42	25	100	9.2(±1.14)	9.2(±1.14)	Ethanol
Western	23/08/2012	601	Winter	-34.42	19.25	25	100	17.12(±2.97)	17.12(±2.97)	Ethanol
Western	17/09/2012	626	Spring	-34.58	18.75	25	88	8.86(±1.65)	7.8(±1.56)	Ethanol
Western	08/10/2012	647	Spring	-32.58	18.08	25	88	5.73(±1.01)	5.04(±0.97)	Ethanol
Western	12/11/2012	682	Spring	-34.75	19.42	25	92	8.52(±4.49)	7.84(4.15)	Ethanol
Overall						**408**	**83.88 (±4.29)**	**6.84(±0.53)**	**5.75(±0.46)**	
Southern	07/03/2011	66	Autumn	-34.68	22.29	25	24	1.67(±0.49)	0.4(±1.83)	Frozen
Southern	29/06/2011	180	Winter	-33.97	25.05	25	56	5.36(±1.07)	3(±0.80)	Frozen
Southern	06/09/2011	249	Spring	-34.60	22.37	25	68	3.82(±1.11)	2.6(±0.83)	Frozen
Southern	03/11/2011	307	Spring	-33.98	25.05	25	60	3.13(±0.82)	1.88(±0.58)	Frozen
Southern	21/12/2011	355	Summer	-34.07	25.80	25	44	3(±0.51)	1.32(±0.37)	Frozen

Table 3.1: Continued

Southern	24/02/2012	420	Summer	-33.96	25.68	25	40	2(±0.54)	0.8(±0.29)	Frozen
Southern	23/03/2012	448	Autumn	-33.85	25.65	25	56	1.79(±0.28)	1(±0.24)	Frozen
Southern	08/05/2012	494	Autumn	-34.00	25.28	25	68	2.35(±0.59)	1.6(±0.46)	Frozen
Southern	26/06/2012	543	Winter	-34.00	25.20	25	64	5.37(±1.65)	3.44(±1.17)	Frozen
Southern	25/07/2012	572	Winter	-34.51	25.38	25	52	1.85(±0.30)	0.96(±0.24)	Ethanol
Southern	21/08/2012	599	Winter	-34.59	25.16	25	76	2.37(±0.34)	1.8(±0.33)	Ethanol
Southern	12/09/2012	621	Spring	-34.59	22.03	25	96	6.96(±1.35)	6.68(1.33)	Ethanol
Southern	08/12/2012	708	Summer	-34.25	24.25	25	20	1.8(±0.58)	0.36(0.18)	Ethanol
Overall						**325**	**55.69(±5.72)**	**3.57(±0.31)**	**1.99(±0.20)**	

Data exploration: spatial variation:

Figures 3.6, 3.7 and 3.8 show that the overall mean prevalence, infection intensity and abundance of "tetracotyle" type metacercariae for all months combined was much higher in *S. sagax* specimens from the putative western stock compared *S. sagax* specimens from the putative southern stock. Mean prevalence off the west coast was 83.88%, while mean prevalence off the south coast was 55.69%. Mean infection intensity was 6.84 and 3.57 parasites per infected fish for the putative western and southern stocks respectively, while abundance averaged 5.75 parasites per fish in the west and 1.99 parasites per fish in the south.

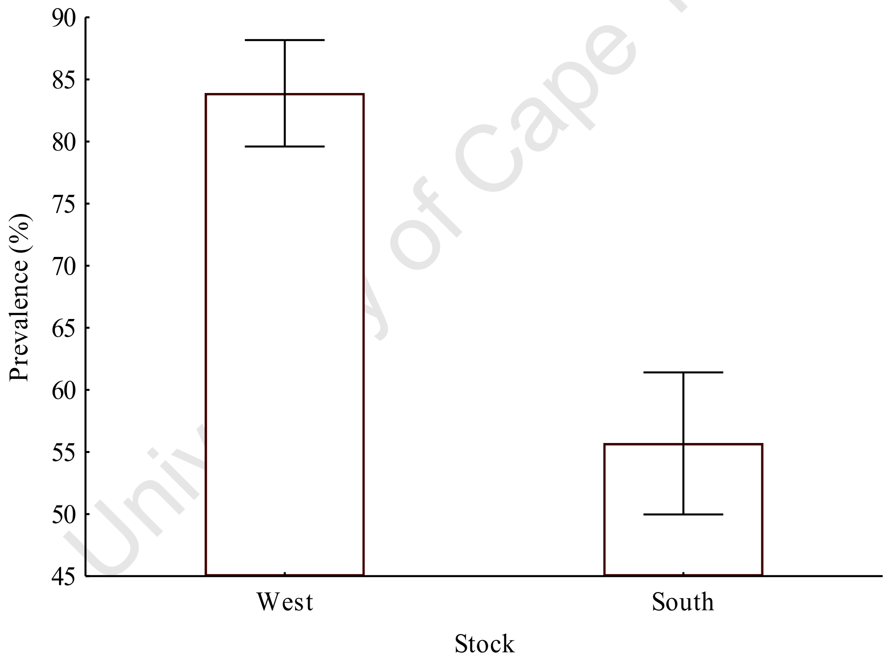

Figure 3.6: Overall mean (±SE) prevalence of "tetracotyle" type metacercariae in *Sardinops sagax* from the putative southern and western stocks off the coast of South Africa in 2011 and 2012.

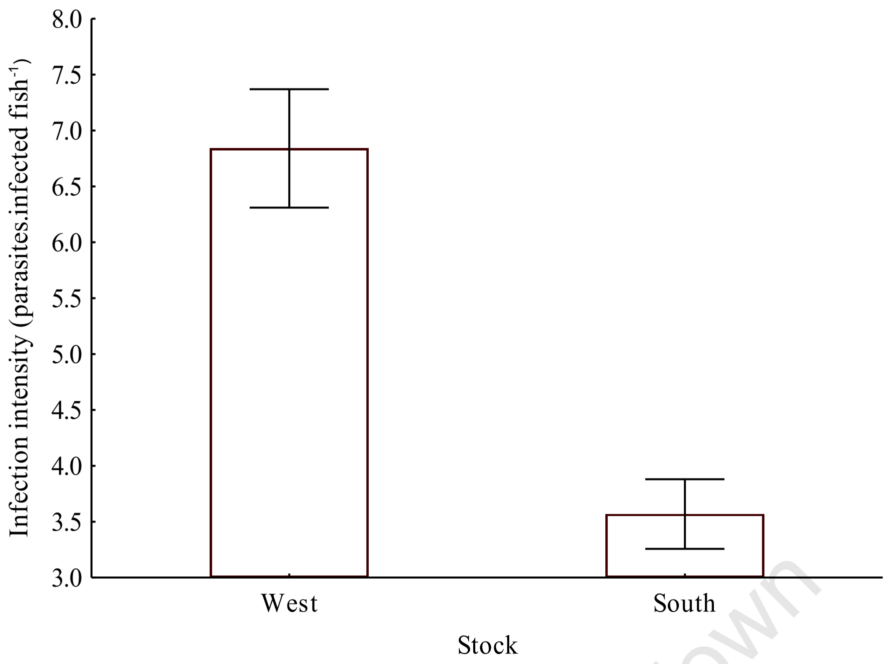

Figure 3.7: Overall mean (±SE) infection intensity of “tetracotyle” type metacercariae in *Sardinops sagax* from the putative southern and western stocks off the coast of South Africa in 2011 and 2012.

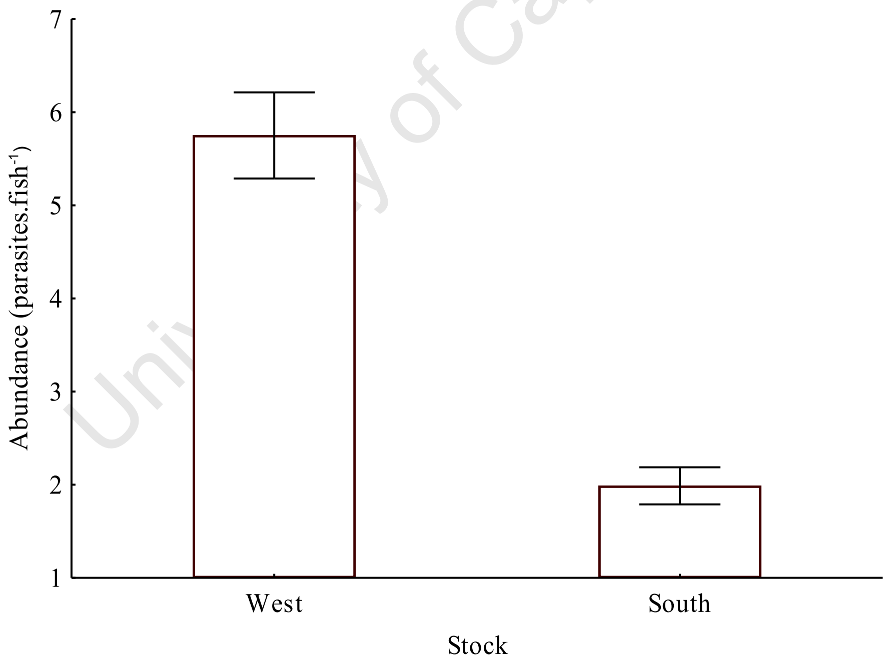

Figure 3.8: Overall mean (±SE) abundance of “tetracotyle” type metacercariae in *Sardinops sagax* from the putative southern and western stocks off the coast of South Africa in 2011 and 2012.

Data exploration: seasonality:

Prevalence:

Figure 3.9 shows a clear seasonal pattern in the prevalence of "tetracotyle" type metacercariae in *S. sagax* from both the putative western and southern stocks. The pattern of seasonality in prevalence seemed to be relatively similar in both stocks, but was to some extent delayed in the southern stock, where peak values for each year in the southern stock occurred slightly after the peak values occurred in the western stock. However, prevalence values seemed to be consistently higher in the putative western stock in comparison to the putative southern stock.

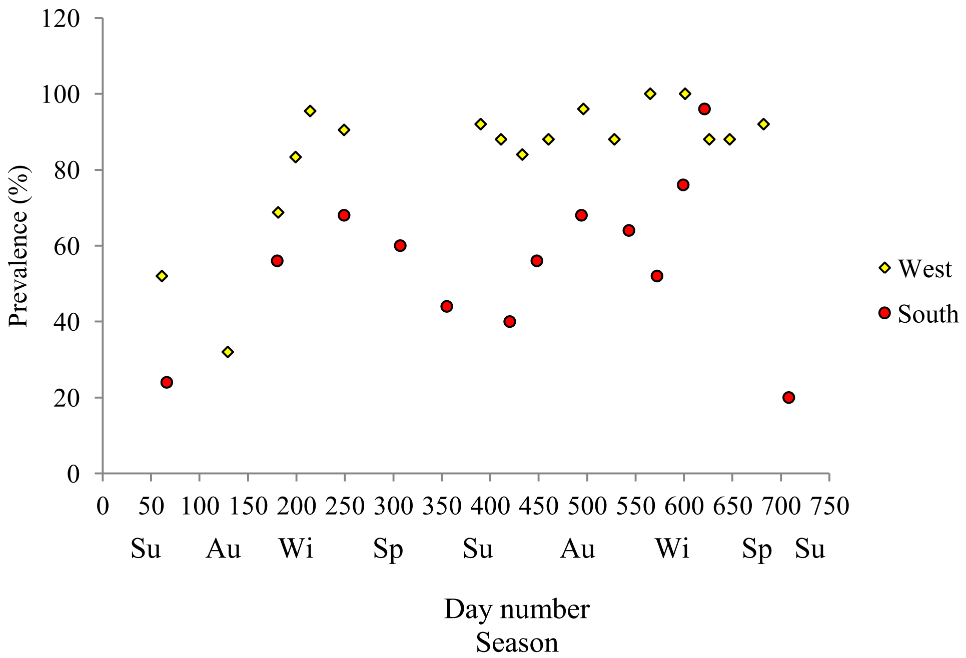

Figure 3.9: Monthly prevalence values of "tetracotyle" type metacercariae infection in *Sardinops sagax* from the putative southern and western stocks off the coast of South Africa, from March 2011 through to December 2012 (Su- summer, Au- autumn, Wi- winter, Sp- Spring).

Infection intensity:

As with prevalence, Figure 3.10 shows that there was clear seasonal variation in the infection intensity of "tetracotyle" type metacercariae in *S. sagax* from both the putative western and southern stocks. Mean infection intensity was similar between fish from the putative western and southern stocks in 2011, but was consistently higher in sardine from the western stock in comparison to sardine from the south in 2012. In the latter year fish in the west that were infected were generally more infected than those fish that are infected in the south. In 2011, mean infection intensity in the putative western stock peaked, during winter, at 3.82 parasites per infected fish, while in the putative southern stock mean infection intensity peaked, also during winter, at 5.36 parasites per infected fish. In 2012, mean infection intensity to the west of Cape Agulhas peaked during winter at 17.12 parasites per infected fish, while to the east of Cape Agulhas mean infection intensity peaked, slightly later during spring, at 6.96 parasites per infected fish.

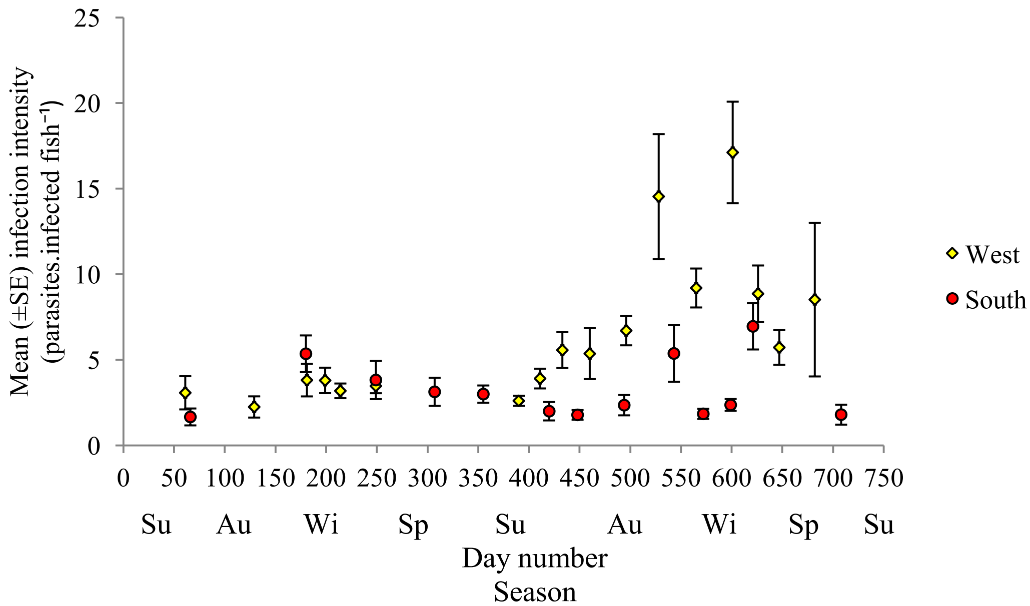

Figure 3.10: Monthly mean (±SE) infection intensities of "tetracotyle" type metacercariae in *Sardinops sagax* from the putative southern and western stocks off the coast of South Africa, from March 2011 through to December 2012 (Su- summer, Au- autumn, Wi- winter, Sp- spring).

Infection intensity of metacercariae was much higher in 2012 than it was in 2011, in both the putative western and southern stocks of *S. sagax*. To standardise for this year effect, the data for each year were normalised by expressing each value as a proportion of the maximum infection intensity value for that year, shown in Figures 3.11, 3.12, 3.13 and 3.14.

When normalising the infection intensity for each year, it becomes clear that the pattern of seasonality was different for each year in both the putative western and southern stock of *S. sagax.* In both the putative western and southern stocks, infection intensity peaked slightly earlier in 2011 compared to 2012. This may be due to a difference in the number of samples collected for each year, and the time interval at which these samples were collected.

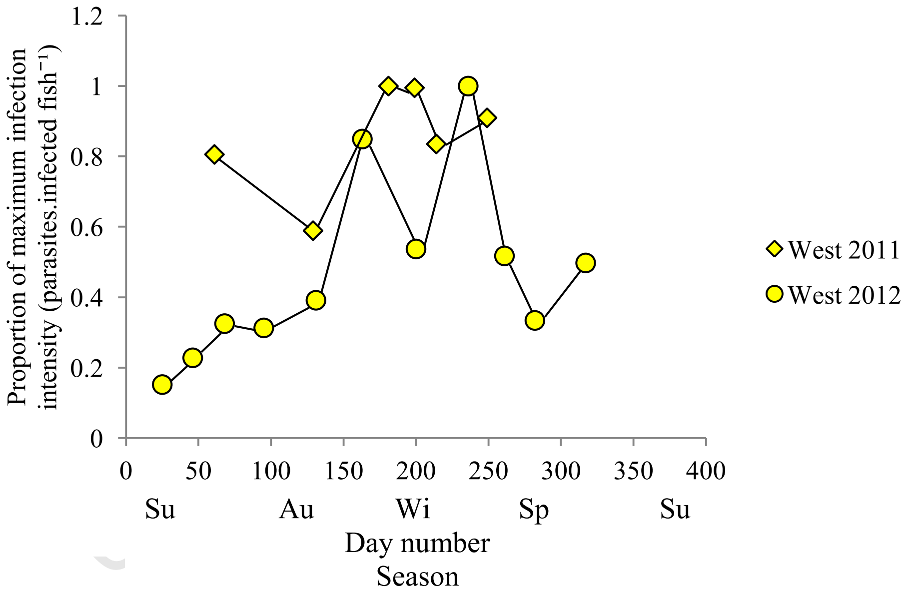

Figure 3.11: Normalised (proportion of maximum) infection intensity of “tetracotyle” type metacercariae in *Sardinops sagax* from the putative western stock off the coast of South Africa, in 2011 and 2012 (Su- summer, Au- autumn, Wi- winter, Sp- spring).

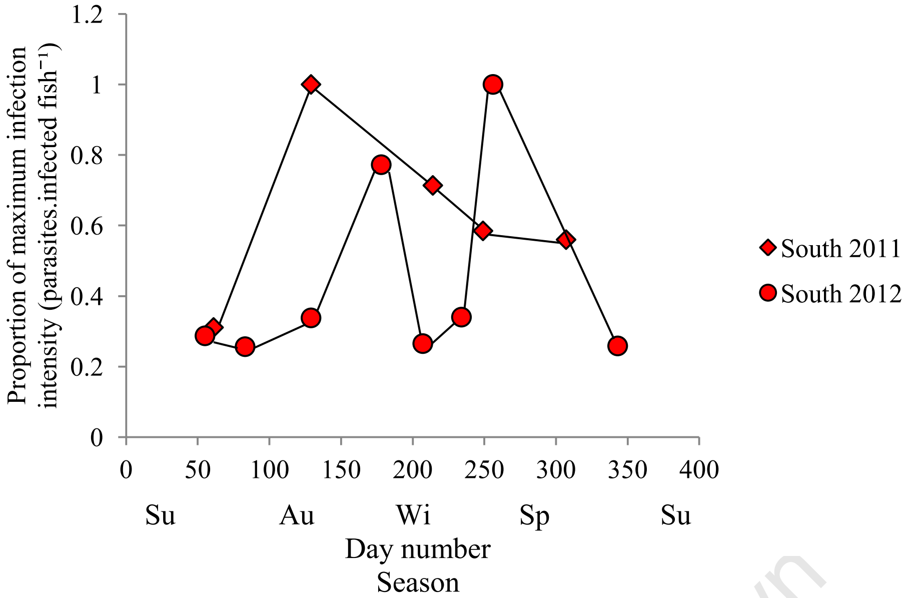

Figure 3.12: Normalised (proportion of maximum) infection intensity of “tetracotyle” type metacercariae in *Sardinops sagax* from the putative southern stock off the coast of South Africa, in 2011 and 2012 (Su- summer, Au- autumn, Wi- winter, Sp- spring).

Figure 3.13 and 3.14 show that, although the pattern in seasonality of infection intensity was different for 2011 and 2012, the pattern was relatively similar for each year in each of the putative stocks. This was particularly true for 2012 where patterns in seasonal variation in infection intensity of metacercariae were closely matched in sardine from the putative western and southern stocks, the only difference being that the seasonal signals seemed to be slightly delayed in the southern stock in comparison to the western stock. The similarity between stocks was not as well defined in 2011, but this may be due to the difference in the number of samples collected in 2011 and the time intervals between sample collections.

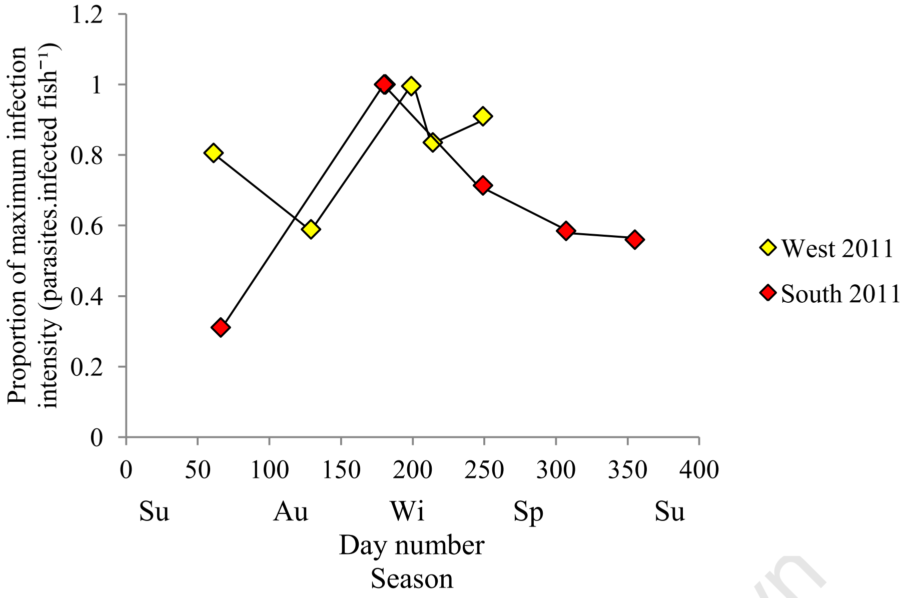

Figure 3.13: Normalised (proportion of maximum) infection intensity of "tetracotyle" type metacercariae in *Sardinops sagax* from the putative western and southern stocks off the coast of South Africa, during 2011 (Su- summer, Au- autumn, Wi- winter, Sp- spring).

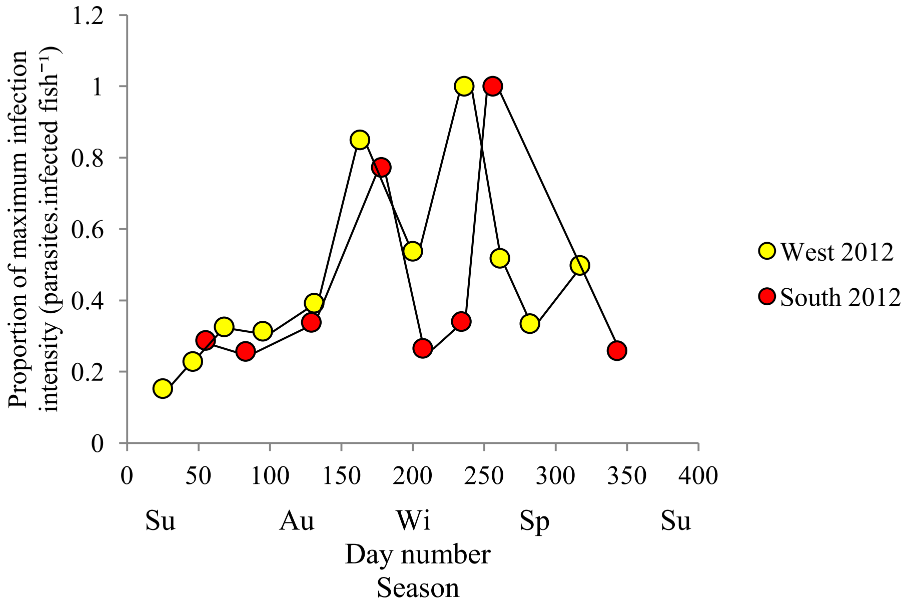

Figure 3.14: Normalised (proportion of maximum) infection intensity of "tetracotyle" type metacercariae in *Sardinops sagax* from the putative western and southern stocks off the coast of South Africa, during 2012 (Su- summer, Au- autumn, Wi- winter, Sp- spring).

Abundance:

As with prevalence and infection intensity, there was a clear monthly and seasonal variation in the abundance of "tetracotyle" type metacercariae in *S. sagax* specimens from both the putative western and southern stocks (Figure 3.15). Mean abundance was typically higher in fish from the putative western stock in comparison to fish from the putative southern stock, and it was only in the winter and early spring of both 2011 and 2012 that abundance values were similar. In 2011, the western stock peaked in mean abundance during winter at 3.18 parasites per fish, while the southern stock peaked, also during winter at 3 parasites per fish. In 2012, the western stock peaked in mean abundance during winter at 17.12 parasites per fish, while the southern stock peaked slightly later, in spring, at 6.68 parasites per fish.

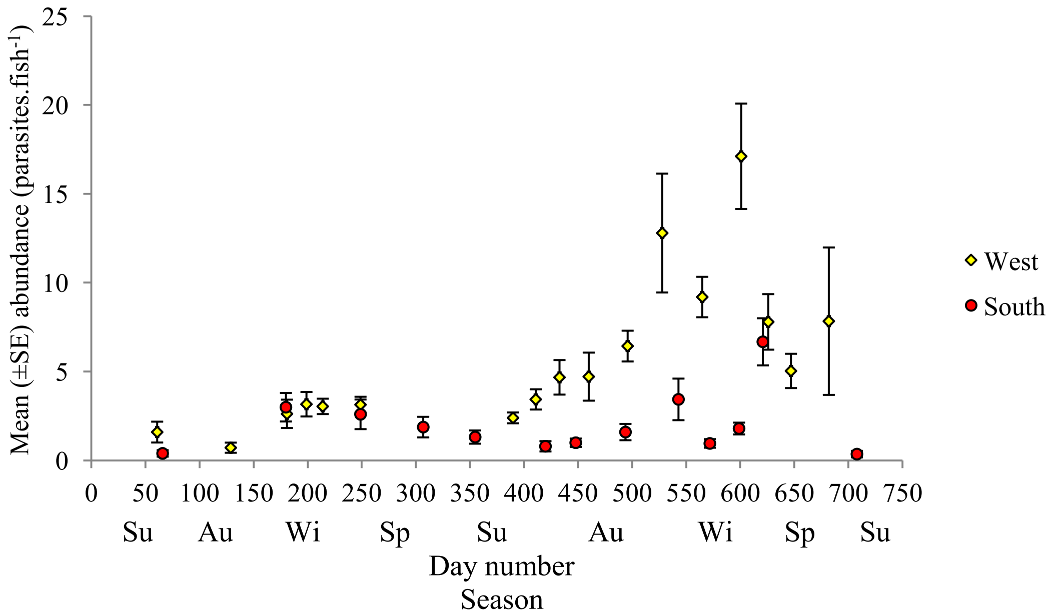

Figure 3.15: Monthly mean (±SE) abundances of "tetracotyle" type metacercariae in *Sardinops sagax* from the putative southern and western stocks off the coast of South Africa, from March 2011 through to December 2012 (Su- summer, Au- autumn, Wi- winter, Sp- spring).

The abundance of metacercariae was much higher in 2012 than it was in 2011, in both the putative western and southern stocks of *S. sagax*. To standardise the data to remove this year effect, the data for each year were normalised by expressing each value as a proportion of the maximum abundance value for that year. This is shown in Figures 3.16, 3.17, 3.18 and 3.19.

By normalising the data for each year, it becomes clear that the pattern of seasonality in the abundance of metacercariae was quite different for 2011 and 2012, in fish from both the west of Cape Agulhas and the east of Cape Agulhas. In fish from both putative stocks, abundance peaked slightly earlier in 2011 compared to 2012 (Figures 3.16 and 3.17). This difference between years may again, however, be an artefact of sampling, since there were fewer samples from 2011, from both the western and southern stocks, which may limit interpretation.

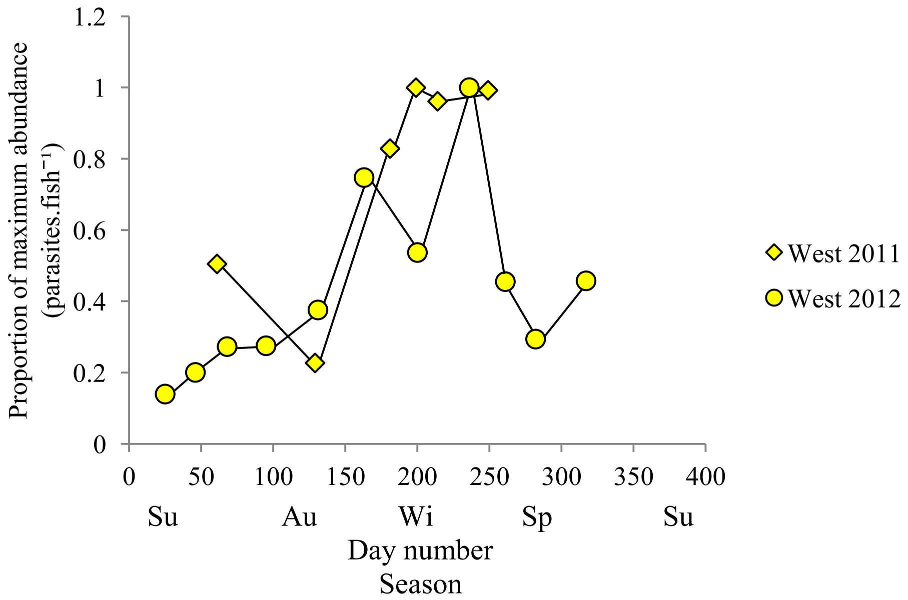

Figure 3.16: Normalised (proportion of maximum) abundance of “tetracotyle” type metacercariae in *Sardinops sagax* from the putative western stock off the coast of South Africa, in 2011 and 2012 (Su- summer, Au- autumn, Wi- winter, Sp- spring).

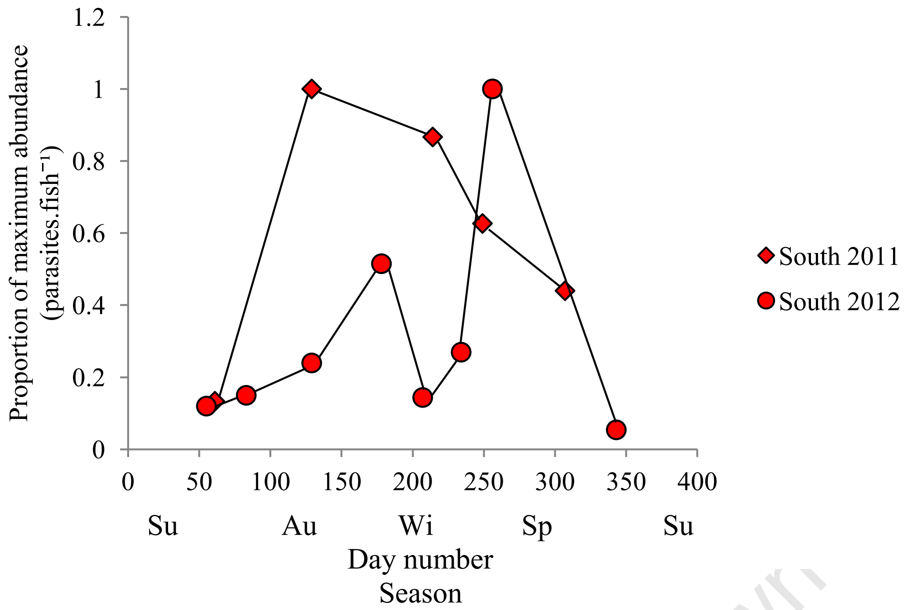

Figure 3.17: Normalised (proportion of maximum) abundance of “tetracotyle” type metacercariae in *Sardinops sagax* from the putative southern stock off the coast of South Africa, in 2011 and 2012 (Su- summer, Au- autumn, Wi- winter, Sp- spring).

When the normalised abundances of “tetracotyle” type metacercariae were separated for each year, it becomes clear that the pattern of seasonality was relatively similar within each putative stock (Figures 3.18 and 3.19). This was particularly true for 2012, where abundances seemed to follow a similar pattern in both the putative western and southern stocks, and was just slightly delayed in the southern stock. The pattern in 2011 was not as clear, but this may be due to the fact that there were fewer samples in 2011 compared to 2012, and, in 2011 samples from the east and the west of Cape Agulhas were not collected at similar time intervals.

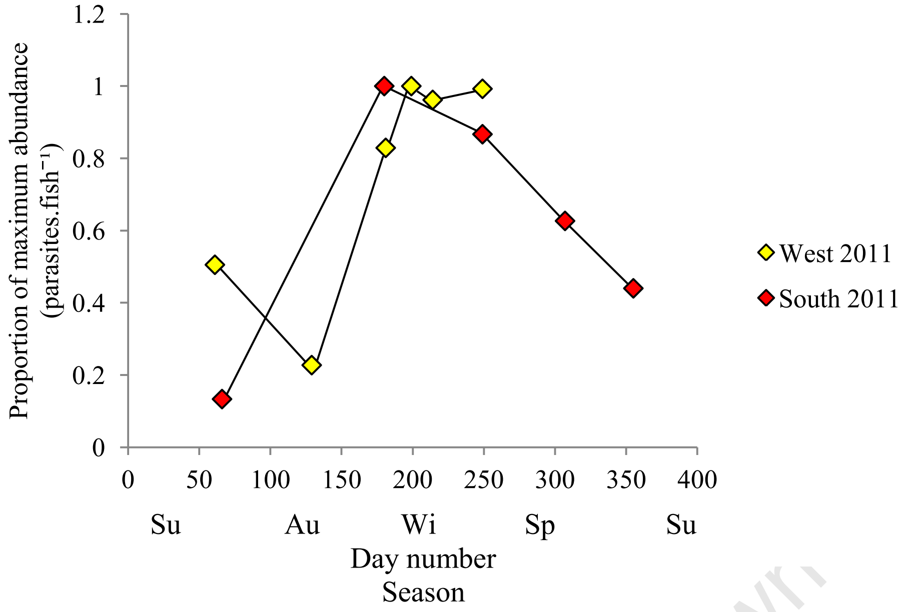

Figure 3.18: Normalised (proportion of maximum) abundance of “tetracotyle” type metacercariae in *Sardinops sagax* from the putative western and southern stocks off the coast of South Africa, during 2011 (Su- summer, Au- autumn, Wi- winter, Sp- spring).

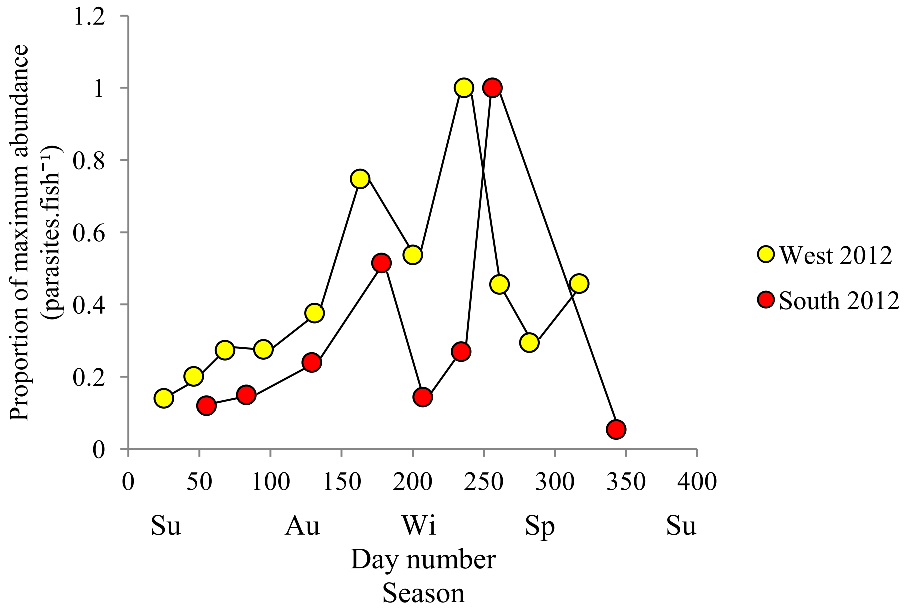

Figure 3.19: Normalised (proportion of maximum) abundance of “tetracotyle” type metacercariae in *Sardinops sagax* from the putative western and southern stocks off the coast of South Africa, during 2012 (Su- summer, Au- autumn, Wi- winter, Sp- spring).

Data exploration: fish size effects on infection intensity and abundance:

Sardinops sagax specimens obtained from the west and south coasts of South Africa, were of a similar size and ranged between 15 and 22cm in caudal length (Figures 3.20 and 3.21). Most of these fish were likely to be adults, as size at 50% sexual maturity ranges from 16.5 to 19.0 cm (van der Lingen *et al.,* 2006a).

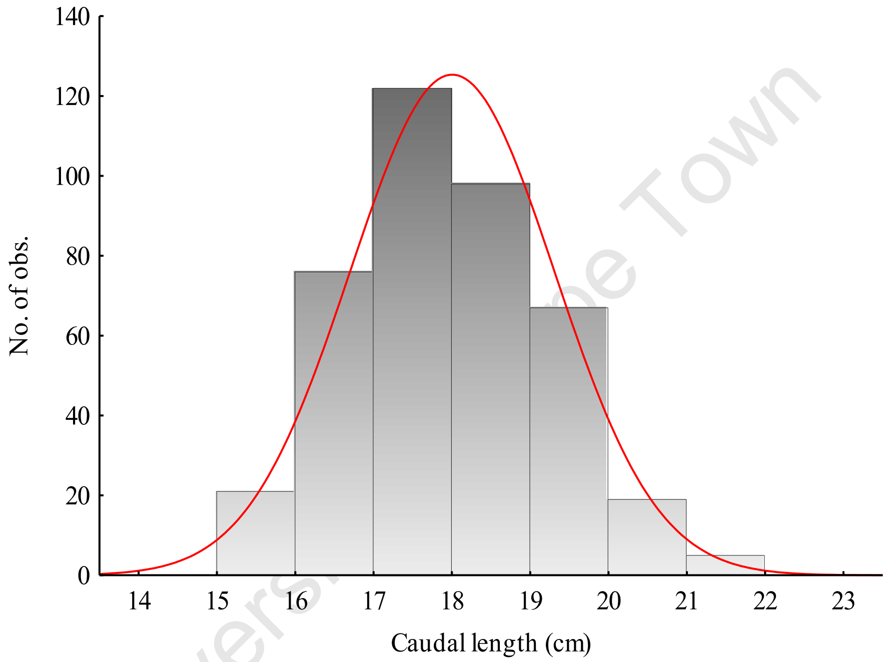

Figure 3.20: Frequency distribution of caudal length for *Sardinops sagax* from the putative western stock off the coast of South Africa in 2011 and 2012. The red line indicates the fit of a normal distribution.

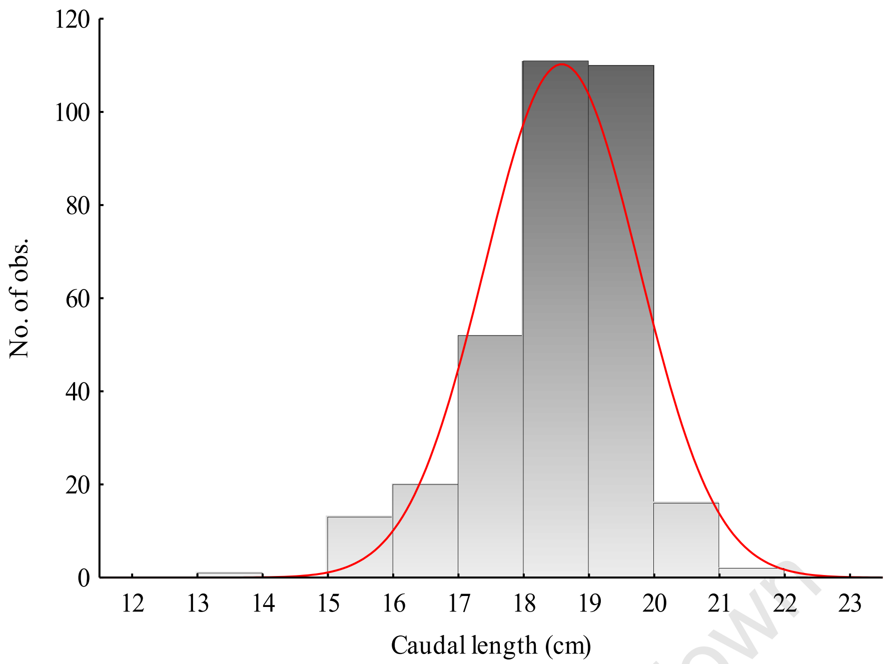

Figure 3.21: Frequency distribution of caudal length for *Sardinops sagax* from the putative southern stock off the coast of South Africa in 2011 and 2012. The red line indicates the fit of a normal distribution.

Infection intensity vs. caudal length:

Highly significant positive linear relationships were found between caudal length and infection intensity of "tetracotyle" type metacercariae in sardine from both western and southern stocks (Figures 3.22 and 3.23), and the parameters of each linear regression are shown in Table 3.2. This therefore implies that the infection intensity of the "tetracotyle" parasite is cumulative with age, and that older, larger fish are more infected than smaller ones.

The comparison of the two slopes from the regression is shown in Table 3.3. The test statistic obtained from the Student's t-test on the slope values falls within the critical region, and so the regression coefficients for the putative southern and western stocks were not significantly different from one another. Therefore, although there was a significant relationship between caudal length and infection intensity, this relationship was not significantly different between the two putative stocks of *S. sagax.*

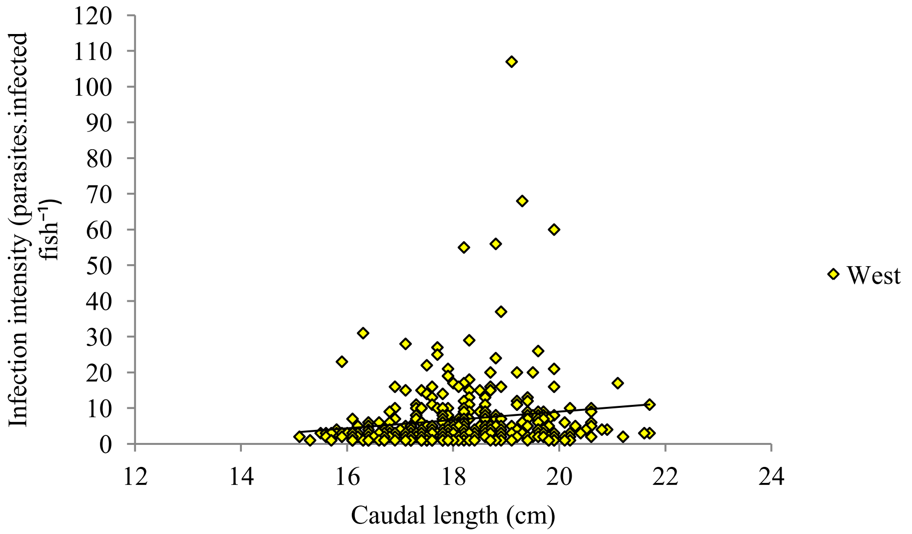

Figure 3.22: Scatterplot and fitted linear relationship between caudal length (cm) and infection intensity of "tetracotyle" type metacercariae in *Sardinops sagax* from the putative western stock off the coast of South Africa in 2011 and 2012.

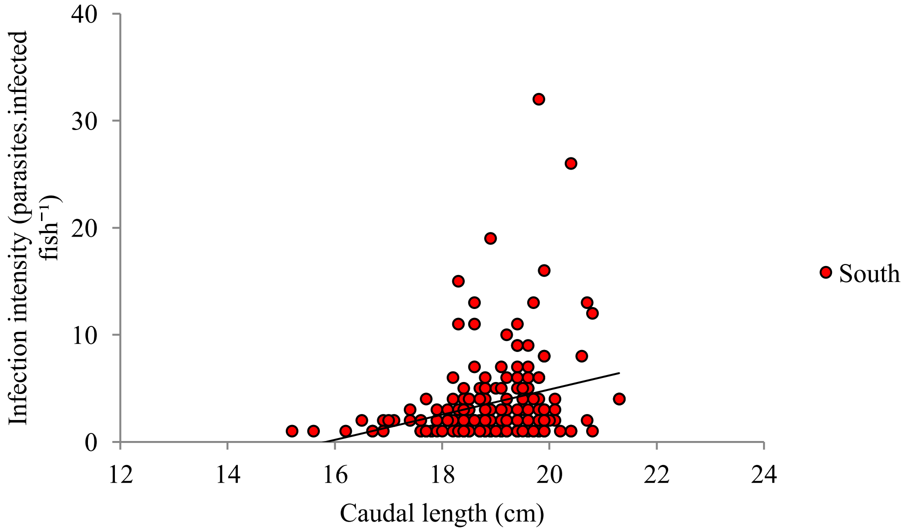

Figure 3.23: Scatterplot and fitted linear relationship between caudal length (cm) and infection intensity of "tetracotyle" type metacercariae in *Sardinops sagax* from the putative southern stock off the coast of South Africa in 2011 and 2012.

Table 3.2: Equations and statistical parameters from the linear regressions between caudal length and infection intensity of "tetracotyle' type metacercariae in *Sardinops sagax* from the putative southern and western stocks off the coast of South Africa in 2011 and 2012.

Stock	Equation	Adjusted R^2	F	p
West	$y = 1.1687x - 14.335$	0.019723	7.880981	<0.05
South	$y = 1.1743x - 18.598$	0.070360	14.62339	<0.05

Table 3.3: Results of a t-test comparing the slopes of the linear regressions between caudal length and infection intensity of "tetracotyle' type metacercariae in *Sardinops sagax* from the putative southern and western stocks off the coast of South Africa in 2011 and 2012 (α= 0.05(2)).

Stock	n	Slope	SE	df	Critical region	t
West	343	1.1687	0.416310	522	t≤-1.965	-0.321
South	181	1.1743	0.307080		t≥1.965	

Abundance vs. caudal length:

As observed for infection intensity, significant positive linear relationships were found between abundance and caudal length in both southern and western stocks (Figures 3.24 and 3.25), and the parameters of the fitted regressions are shown in Table 3.4. This implies that the abundance of "tetracotyle" type metacercariae is cumulative in *S. sagax*, where older, larger fish have a higher abundance of parasites than younger, smaller fish.

The comparison of the two slopes from the fitted regressions are shown in Table 3.5. The test statistic obtained from the Student's t-test again falls within the critical region, and so the regression coefficients for the putative southern and western stocks were not significantly different from one another. This implies that, as with infection intensity, although there was a significant relationship between abundance and caudal length, this relationship was not different between the putative western and southern stocks of *S. sagax.*

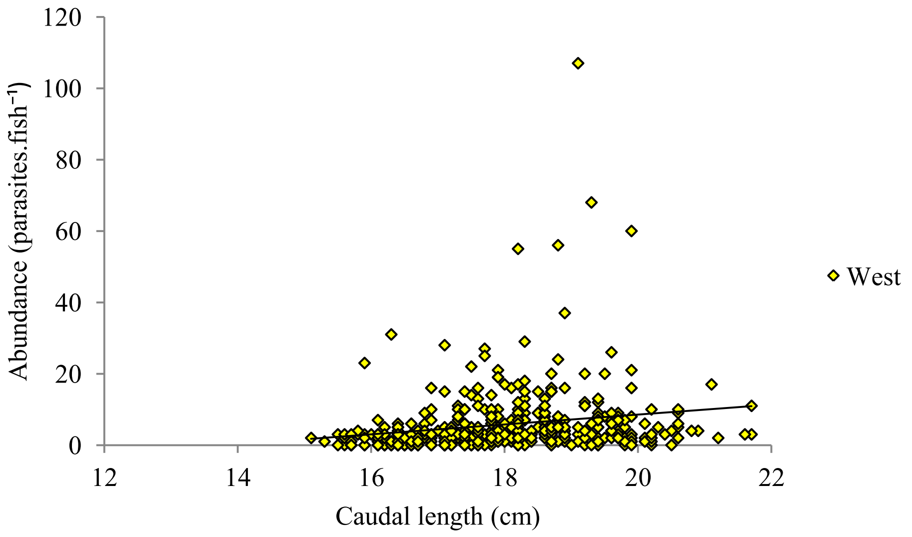

Figure 3.24: Scatterplot and fitted linear relationship between caudal length (cm) and abundance of “tetracotyle” type metacercariae in *Sardinops sagax* from the putative western stock off the coast of South Africa in 2011 and 2012.

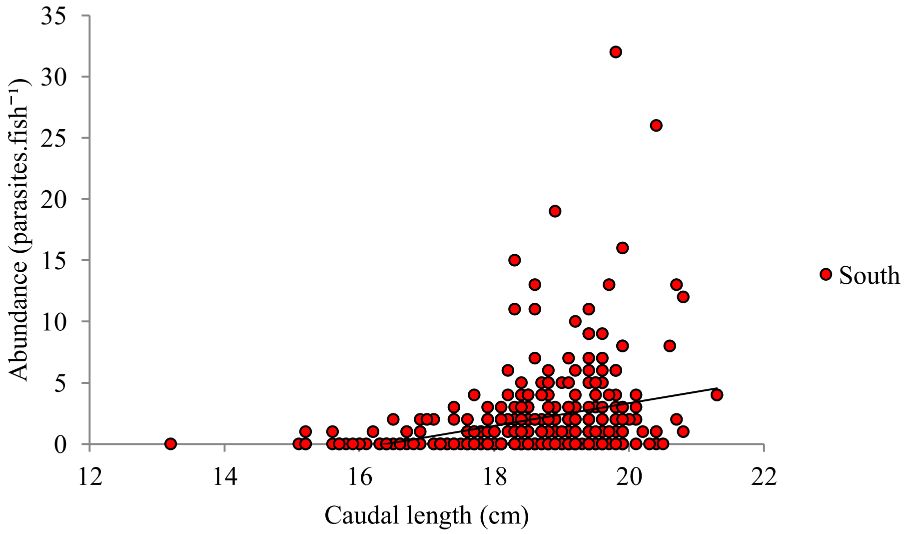

Figure 3.25: Scatterplot and fitted linear relationship between caudal length and abundance of “tetracotyle” type metacercariae in *Sardinops sagax* from the putative southern stock off the coast of South Africa in 2011 and 2012.

Table 3.4: Equations and statistical parameters from the linear regressions between caudal length and abundance of “tetracotyle’ type metacercariae in *Sardinops sagax* from the putative southern and western stocks off the coast of South Africa in 2011 and 2012.

Stock	**Equation**	**Adjusted R^2**	**F**	**p**
West	$y = 1.39x - 19.245$	0.035009	15.76568	<0.05
South	$y = 0.9265x - 15.199$	0.089135	32.70568	<0.001

Table 3.5: Results of a t-test comparing the slopes of the linear regressions between caudal length and abundance of "tetracotyle' type metacercariae in *Sardinops sagax* from the putative southern and western stocks off the coast of South Africa in 2011 and 2012 (α= 0.05(2))

Stock	n	Slope	SE	df	Critical region	t
West	408	1.39	0.350066	731	t≤-1.963	1.162
South	325	0.9265	0.162015		t≥1.963	

Statistical analyses:

Prevalence:

Because prevalence had a binomial distribution with only two responses- either infected or uninfected, normality was not tested for. A Generalized Linear Model with a binomial distribution was therefore fitted, and the logit link function was used. All factors were found to make a significant contribution to the model, as indicated by the AIC, and so none were excluded from the analysis.

An analysis of deviance was conducted (Table 3.6), and showed that majority of the factors were highly significant, where $p < 0.001$. Using the null and residual deviance values obtained from the analysis of deviance, the pseudo R^2 value was calculated. R^2 was found to equal 0.2311, and so the binomial model with the logit link function explained 23.11% of the variance or deviance in the prevalence of the "tetracotyle" parasite in *S. sagax* collected off the coast of South Africa. Each predictor contributed a certain percentage to this final deviance value. These contributions are also shown in Table 3.6. Stock explained the majority of the variance (39.34%) in the prevalence of the "tetracotyle" parasite. This contribution was almost double that of the next contributor, season, which explained 20.72%. Year and length were the next most important predictors, and had a similar contribution to season, explaining 17.61 and 15.19% of the deviance in prevalence, respectively. The stock-season interaction was the least important contributor, explaining 7.14% of the variance seen in prevalence of infection.

Table 3.6: Results of the analysis of deviance obtained from the binomial model fitted to the prevalence of "tetracotyle" type metacercariae found in *Sardinops sagax* specimens from the putative western and southern stocks off the coast of South Africa in 2011 and 2012.

	Residual Df	Residual deviances	ΔDeviance	p-value	% deviance explained
NULL	732	876.28			
Stock	731	804.16	-72.11	<0.001	39.34
Season	728	766.19	-37.98	<0.001	20.72
Year	727	733.91	-32.28	<0.001	17.61
Log(length)	726	706.06	-27.85	<0.001	15.19
Stock*season	723	692.96	-13.09	<0.01	7.14

Predicted prevalence values by season for sardine from the putative western and southern stocks are shown in Figure 3.26. The differences in prevalence between fish from the putative western and southern stocks as well as the differences in prevalence between seasons, as predicted by the binomial model, are clear. Prevalence values were normalised for year and length, where a mean caudal length of 18.3cm was used. Predicted prevalence of the "tetracotyle" parasite in the putative western stock of sardine was significantly higher in comparison to the putative southern stock, where $p < 0.001$. Within this pattern, each stock showed its own significant seasonal variance. In the putative western stock of sardine, predicted prevalence was high year round and peaked in winter at 71.47%. Predicted prevalence in the western stock in summer was higher than in autumn, but slightly lower than in spring. In the putative southern stock, prevalence increased steadily and peaked in spring, slightly later than the western stock, at 69.57%. The prevalence values for spring were similar in both putative stocks, and were not significantly different from one another. Due to the significant stock-season interaction, separate predicted values for stock and season were not generated.

Differences in the prevalence of “tetracotyle” type metacercariae between the years used in this study, as predicted by the binomial model are shown in Figure 3.27. The predictive data for year was normalised for stock, season and length (18.3cm). Prevalence of the “tetracotyle” parasite was significantly higher in 2012 than in 2011, where $p < 0.001$. Overall prevalence was 68.38% in 2011, while in 2012 it was 71.47%.

The increase in prevalence of “tetracotyle” type metacercariae with increasing caudal length of *S. sagax* as predicted by the binomial model is shown in Figure 3.28. The data set was normalised for stock, season and year. The prevalence of the “tetracotyle” parasite within both putative stocks showed an almost linear increase with an increase in caudal length, and so this positive relationship supports the hypothesis of the cumulative effect of the parasite on sardine, with age.

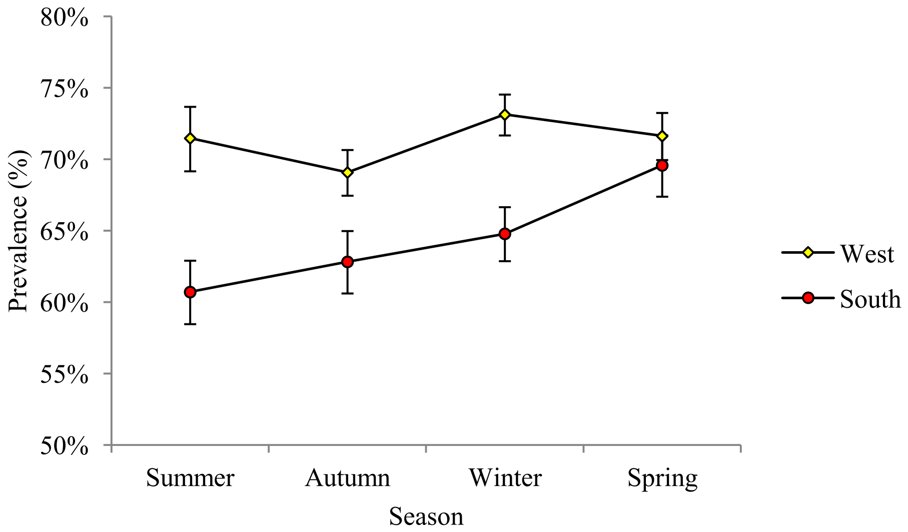

Figure 3.26: Predicted values (± 95% CI) of the prevalence of “tetracotyle” type metacercariae in *Sardinops sagax* from both the putative western and southern stocks off the coast of South Africa, over a period of four seasons. Values have been normalised for year and length (18.3cm caudal length).

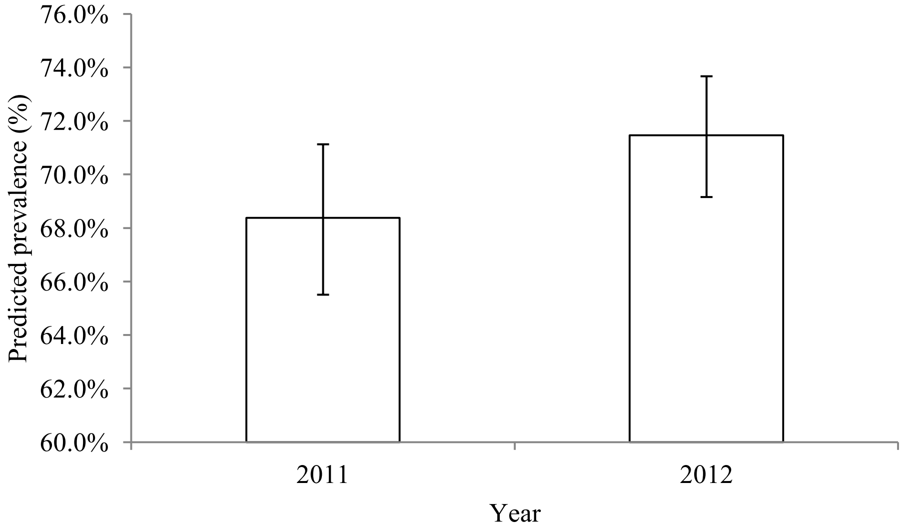

Figure 3.27: Predicted prevalence (±95% CI) of “tetracotyle” type metacercariae in *Sardinops sagax* collected off the coast of South Africa for 2011 and 2012. Values have been normalised for stock, season and length (18.3cm caudal length)

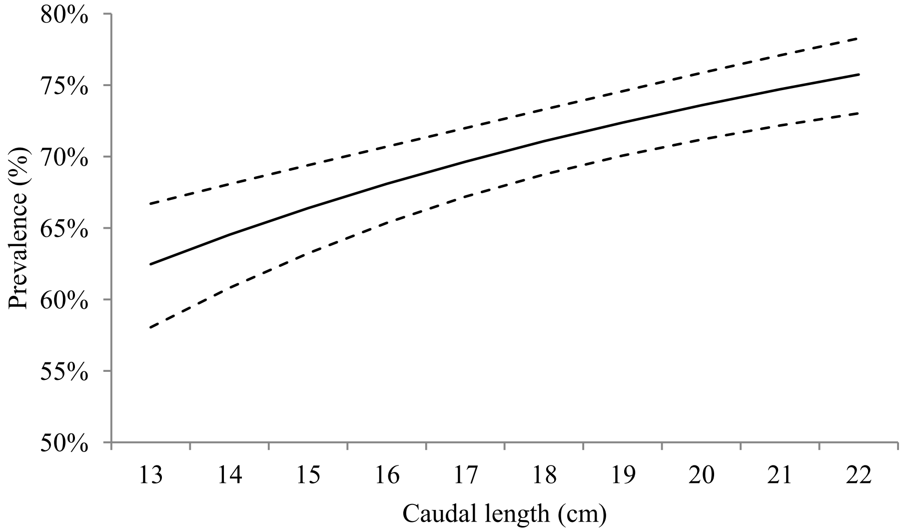

Figure 3.28: Predicted prevalence of “tetracotyle” type metacercariae in *Sardinops sagax* specimens, collected off the coast of South Africa, with increasing caudal length, where the dashed lines represent 95% upper and lower confidence intervals. Values have been normalised for stock, year and season.

Infection intensity:

The frequency distributions shown in Figures 3.29 and 3.30 show that the infection intensity data for both the putative southern and western stocks of *S. sagax* are not normally distributed, due to the very long tail to the right of the data. The infection intensity data for both putative stocks therefore did not fulfil the assumptions of a parametric General Linear Model.

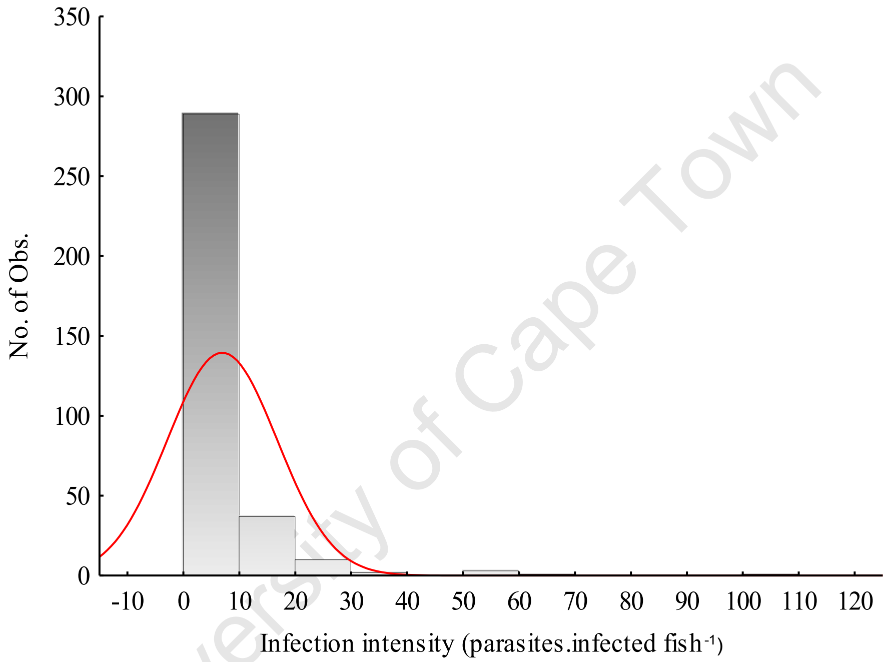

Figure 3.29: Frequency distribution of infection intensity of "tetracotyle" type metacercariae in *Sardinops sagax* specimens from the putative western stock off the coast of South Africa in 2011 and 2012. The red line indicates the fit of a normal distribution.

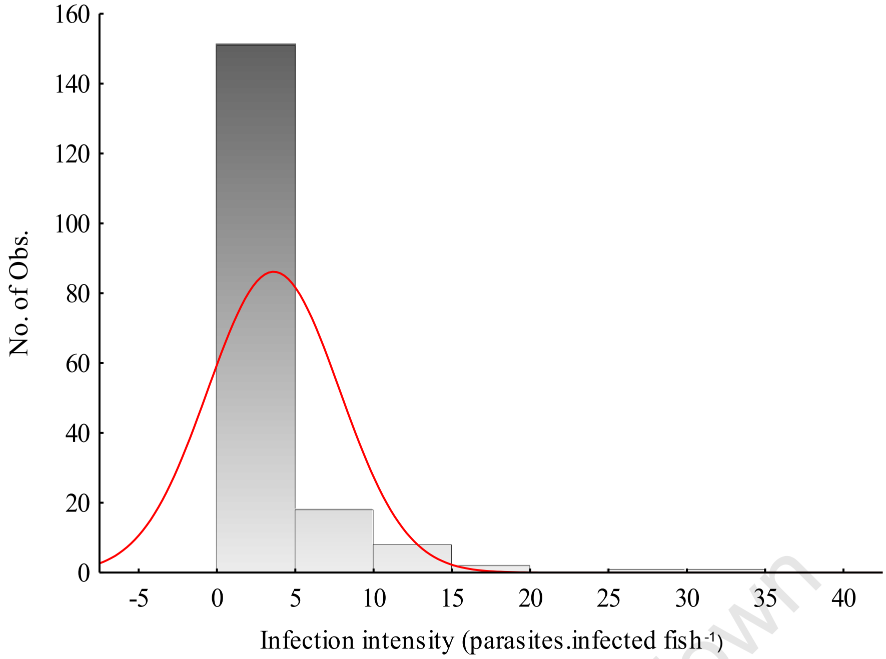

Figure 3.30: Frequency distribution of infection intensity of "tetracotyle" type metacercariae in *Sardinops sagax* specimens from the putative southern stock off the coast of South Africa in 2011 and 2012. The red line indicates the fit of a normal distribution.

The infection intensity data for both the putative western and southern stocks was therefore fitted to a Generalized Linear Model. Because infection intensity was a non-zero data set, where all values were equal to or greater than one, a zero-truncated model was used in order to account for this. Firstly, a zero-truncated Poisson distribution was fitted. All factors were found to have a significant contribution to the model, as indicated by the AIC, and so none were excluded from the analysis. The dispersion of the data was calculated, and was found to equal 7.338, a very high value. In order for a set of data to be considered to be dispersed over an adequate range, the ratio of dispersion needs to equal approximately 1 (Zuur *et al.,* 2009). Since this was not the case, the infection intensity data was considered to be highly over-dispersed.

A zero-truncated negative binomial distribution was therefore fitted to the data. Again, all factors were found to have a significant contribution to the model, as indicated by the AIC, and so none were excluded from the analysis.

An analysis of deviance was conducted (Table 3.7), and showed that all factors were highly significant, where $p < 0.001$. Using the null and residual deviance values obtained from the analysis of deviance, the pseudo R^2 value was calculated. R^2 was found to equal 0.3249, and so the negative binomial model explained 32.49% of the variance in the infection intensity of the "tetracotyle" type metacercariae found in sardine collected off the west and south coasts of South Africa. Each predictor contributed a certain percentage to the overall fit and these contributions are also shown in Table 3.7. In contrast to prevalence, stock and season contributed more equally to the deviance in infection intensity of the "tetracotyle" parasite. In fact, season contributed slightly more to the deviance than stock, where season contributed 29.64% and stock contributed 27.78%. Year was the next most important contributor where it explained 20.23% of the variance in infection intensity. Length and the stock-season interaction were the least important contributors, each explaining a similar percentage of the deviance in infection intensity, where the stock-season interaction explained 12.53% and length explained 9.83%.

Table 3.7: Results of the analysis of deviance obtained from the negative binomial model fitted to the infection intensity of "tetracotyle" type metacercariae found in *Sardinops sagax* specimens from the putative western and southern stocks off the coast of South Africa in 2011 and 2012.

	Residual Df	**Residual deviances**	**ΔDeviance**	**p-value**	**% deviance explained**
NULL	523	736.34			
Stock	522	669.88	-66.46	<0.001	27.78
Season	519	598.96	-70.91	<0.001	29.64
Year	518	550.57	-48.40	<0.001	20.23
Log(length)	517	527.06	-23.51	<0.001	9.83
Stock*season	514	497.09	-29.97	<0.001	12.53

Predicted infection intensity values by season for sardine from the putative western and southern stocks are shown in Figure 3.31. The data were normalised for year and length, where a mean caudal length of 18.3cm was used. The differences in infection intensities between stocks as well as between seasons are clear. Each putative stock showed its own seasonal pattern with significant seasonal variance. In fish from the putative western stock, predicted infection intensity was high and gradually increased from summer through to winter, where it peaked at 11.25 parasites per infected fish, thereafter decreasing in spring. In the putative southern stock, predicted infection intensity was significantly lower than fish from the putative western stock, where $p < 0.001$. Predicted infection intensity in fish to the east of Cape Agulhas was similar in summer and autumn, and then increased gradually to peak, slightly later than the putative western stock, in spring at 6.31 parasites per infected fish. Predicted infection intensity values were similar for both stocks in summer and in spring. Due to the significant stock-season interaction, separate predicted values for stock and season were not generated.

Differences in the infection intensity of “tetracotyle type metacercariae between each year used in this study, as predicted by the zero-truncated negative binomial model, are shown in Figure 3.32. Predicted values for year were normalised for stock, season and length, where again, an average length of 18.3cm was used. The infection intensity of the “tetracotyle” parasite was significantly greater in 2012 in comparison to 2011, where $p < 0.001$, with infection intensity in 2012 being equal to 3.83 parasites per infected fish, and infection intensity in 2011 being equal to 2.51 parasites per infected fish.

The infection intensity of the “tetracotyle” parasite increased exponentially with increasing fish caudal length, predicted by the zero-truncated negative binomial model (Figure 3.33). The data set was normalised for stock, season and year. This positive relationship with caudal length further supports the hypothesis that the "tetracotyle" parasite shows a cumulative infection in sardine, with increasing age.

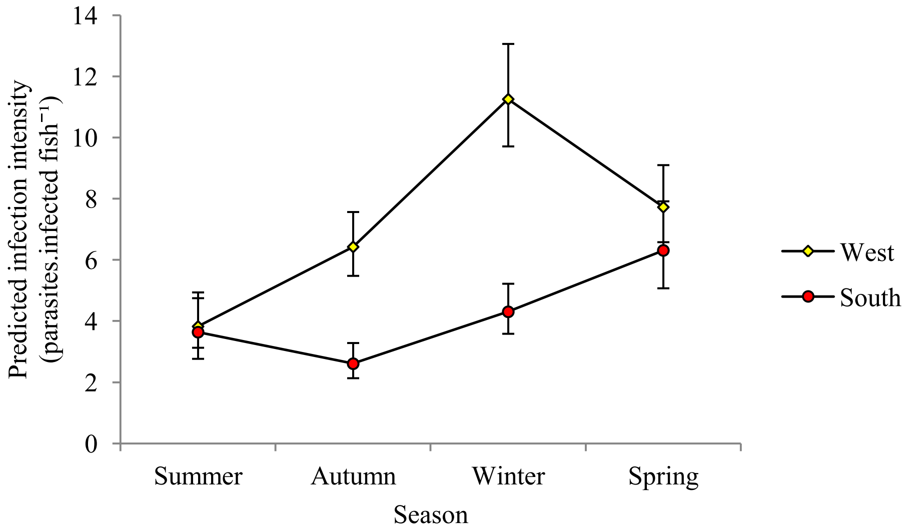

Figure 3.31: Predicted values (± 95% CI) of infection intensity of "tetracotyle" type metacercariae in *Sardinops sagax* from both the putative western and southern stocks off the coast of South Africa, over a period of four seasons. Values have been normalised for year and length (18.3cm caudal length).

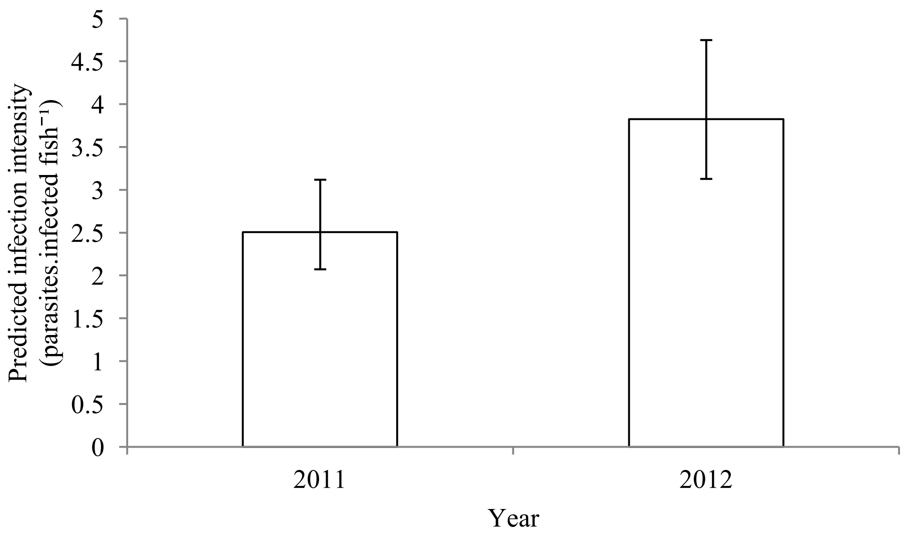

Figure 3.32: Predicted infection intensities (±95% CI) of "tetracotyle" type metacercariae in *Sardinops sagax* collected off the coast of South Africa for 2011 and 2012. Values have been normalised for stock, season and length (18.3cm caudal length).

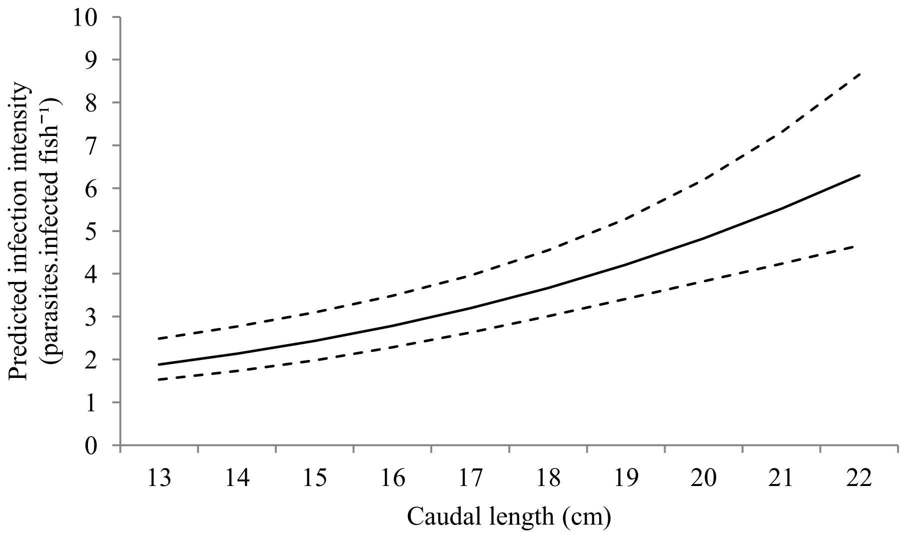

Figure 3.33: Predicted infection intensities of "tetracotyle" type metacercariae in *Sardinops sagax* specimens, collected off the coast of South Africa, with increasing caudal length, where the dashed lines represent 95% upper and lower confidence intervals. Values have been normalised for stock, year and season.

Abundance:

The frequency distributions shown in Figures 3.34 and 3.35 show that the abundance data for both the putative southern and western stocks of *S. sagax* are not normally distributed, again because of the long tail to the right of the data. The parasite abundance data from both putative stocks therefore again did not fulfil the assumptions of a parametric General Linear Model.

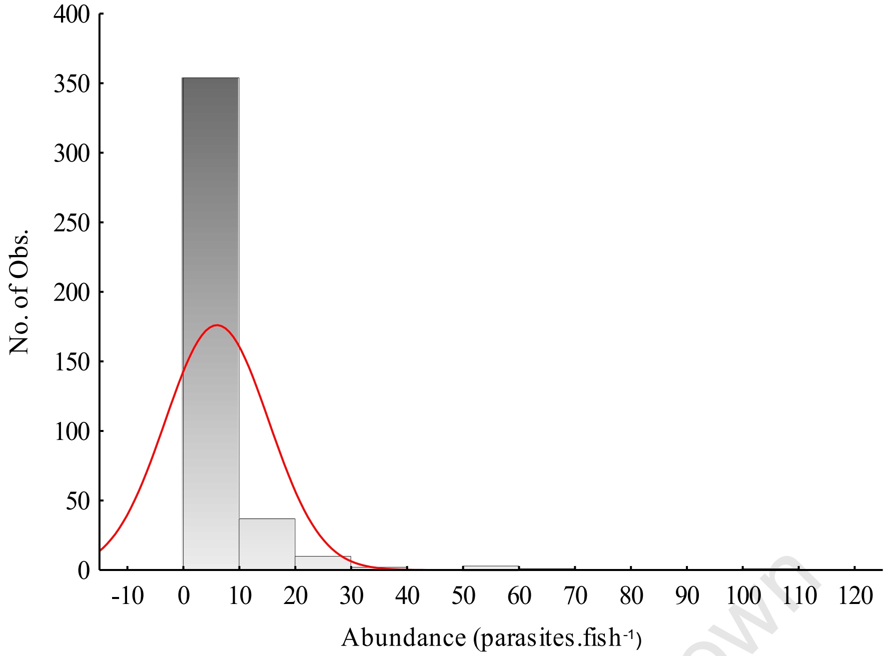

Figure 3.34: Frequency distribution of abundance of “tetracotyle” type metacercariae in *Sardinops sagax* specimens from the putative western stock off the coast of South Africa in 2011 and 2012. The red line indicates the fit of a normal distribution.

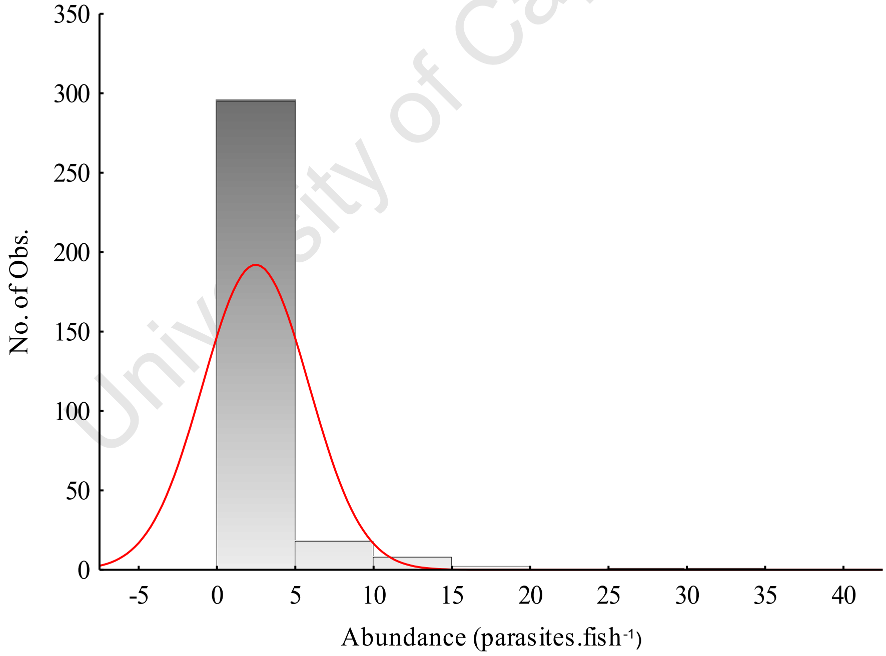

Figure 3.35: Frequency distribution of abundance of “tetracotyle” type metacercariae in *Sardinops sagax* specimens from the putative southern stock off the coast of South Africa in 2011 and 2012. The red line indicates the fit of a normal distribution.

As with infection intensity, the abundance data for both the putative western and southern stocks was therefore fitted to a Generalized Linear Model with firstly, a Poisson distribution. All factors were found to have a significant contribution to the Poisson model, as indicated by the AIC, and so none were excluded. The dispersion of the total data set was calculated and was found to equal 7.074, again, a very high value. Since the ratio of dispersion was not equal to approximately 1, the abundance data was considered to be highly over-dispersed.

A negative binomial distribution was therefore fitted. Again, all factors were found to have a significant contribution to the negative binomial model, as indicated by the AIC, and therefore none were excluded.

An analysis of deviance was conducted (Table 3.8), and showed that all of the factors were highly significant, where $p < 0.001$. Using the null and residual deviance values obtained in the analysis of deviance, the pseudo R^2 value was calculated. R^2 was found to be equal to 0.3456, and so the negative binomial model that was fitted to the abundance data was found to explain 34.56% of the deviance found in the abundance of the "tetracotyle" parasite. Each predictor variable explained a certain percentage of the total deviance. These contributions are also shown in Table 3.8. Again stock explained majority of the variance in abundance (36.58%) of the "tetracotyle" parasite. Season was the next most important contributor to the variation in abundance, where it explained 27.39%. It contributed slightly more to the variance in abundance in comparison to its contribution to prevalence, but it contributed slightly less in comparison to its contribution to infection intensity. In contrast stock contributed slightly less to the variance in abundance compared to the variance in prevalence, but it contributed more than its contribution to infection intensity. Year, length and the stock-season interaction explained less of the variance.

Table 3.8: Results of the analysis of deviance obtained from the negative binomial model fitted to the abundance of “tetracotyle” type metacercariae found in *Sardinops sagax* specimens from the putative western and southern stocks off the coast of South Africa in 2011 and 2012.

	Residual Df	Residual deviances	ΔDeviance	p-value	% deviance explained
NULL	732	1207.31			
Stock	731	1054.67	-152.63	<0.001	36.58
Season	728	940.38	-114.30	<0.001	27.39
Year	727	873.11	-67.27	<0.001	16.12
Log(length)	726	820.93	-52.18	<0.001	12.50
Stock*season	723	790.06	-30.87	<0.001	7.40

The predicted parasite abundance values by season are shown separately for putative western and southern fish (Figure 3.36). The predicted abundance values were normalised for year and length, where a mean caudal length of 18.3cm was used. Again, overall, fish from the putative western stock had a significantly greater abundance of “tetracotyle” type metacercariae, than did those from the putative southern stock, where $p < 0.001$. Each putative stock displayed its own seasonal pattern and showed significant seasonal variation in abundance. In the putative western stock, parasite abundance increased from summer until it peaked in winter, at 10.55 parasites per fish, after which it declined. In the putative southern stock parasite abundance was similar for summer and autumn, and then increased gradually to peak in spring at 4.53 parasites per fish. Spring was the only season in which the abundances of “tetracotyle” in both stocks were similar and were not significantly different from one another. Due to the significant stock-season interaction, separate predicted values for stock and season were not generated.

Differences in the abundances of “tetracotyle type metacercariae between each year used in this study, as predicted by the negative binomial model, are shown in Figure 3.37. Predicted values for year were normalised for stock, season and length, where again, an average length of 18.3cm was used. The abundance of the "tetracotyle" parasite was significantly greater in 2012 in comparison to 2011, where $p < 0.001$, with abundance in 2012 being approximately double that in 2011.

"Tetracotyle" type metacercariae abundance increased exponentially with increasing fish caudal length, predicted by the negative binomial model (Figure 3.38). The data set was normalised for stock, season and year. This further supports the hypothesis that the "tetracotyle" parasite shows a cumulative infection in sardine, with increasing age.

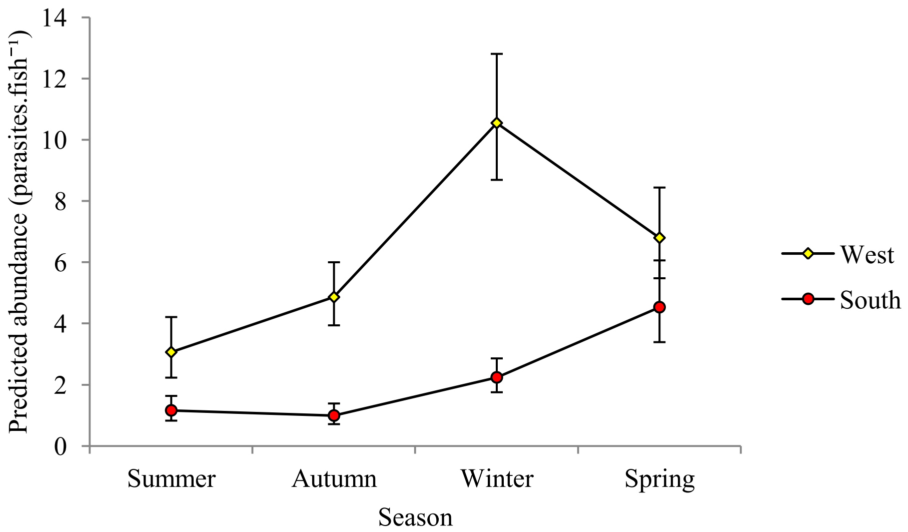

Figure 3.36: Predicted values (± 95% CI) of abundance of "tetracotyle" type metacercariae in *Sardinops sagax* from both the putative western and southern stocks off the coast of South Africa, over a period of four seasons. Values have been normalised for year and length (18.3cm caudal length).

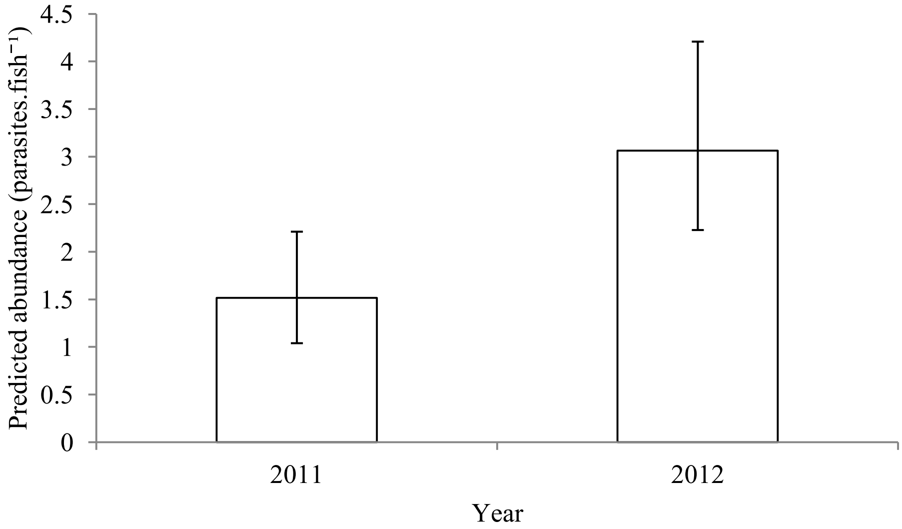

Figure 3.37: Predicted abundance (±95% CI) of “tetracotyle” type metacercariae in *Sardinops sagax* collected off the coast of South Africa for 2011 and 2012. Values have been normalised for stock, season and length (18.3cm caudal length).

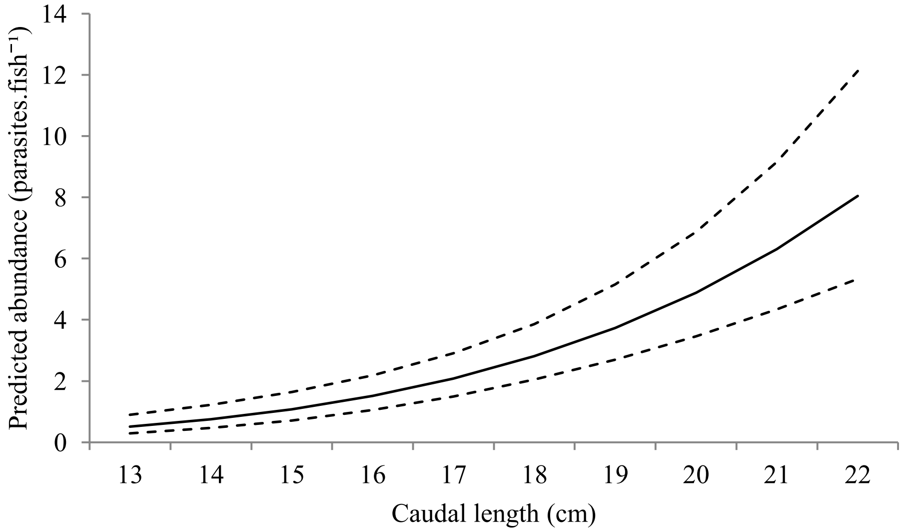

Figure 3.38: Predicted abundance of “tetracotyle” type metacercariae in *Sardinops sagax* specimens, collected off the coast of South Africa, with increasing caudal length, where the dashed lines represent 95% upper and lower confidence intervals. Values have been normalised for stock, year and season.

CHAPTER 4: Discussion

The sardine *Sardinops sagax* is a vital pelagic resource in South African waters, where it is important, both economically and ecologically. Preservation and sustainability of the resource is therefore of utmost importance, and so effective management is essential.

If true, the occurrence of more than one stock of sardine off the coast of South Africa will have severe implications for management of the fishery. Management strategies will have to be altered so as to take account of the three subpopulations, possibly leading to separate regulatory mechanisms for each stock. Many studies support the multiple stock hypothesis. This, coupled with the fact that recently there has been a decline in sardine biomass and catches in South African waters, particularly off the west coast, has led to the investigation of various multiple stock alternatives and their possible inclusion in the operational management procedure used in the management of the South African sardine resource (de Moor & Butterworth, 2012).

The use of "tetracotyle" type metacercariae as a biological tag to distinguish different stocks of sardine off the coast of South Africa was suggested by Reed *et al.* (2012), because it seemed to fulfil majority of the criteria of a successful tag. This method of stock identification has a variety of advantages and has been widely used elsewhere. Results from the study by Reed *et al.* (2012) showed higher prevalence of the "tetracotyle" parasite in *S. sagax* specimens from the west coast of South Africa compared to those from the south coast, supporting the multiple stock hypothesis. Further research, with larger sample sizes, to examine the geographical distribution and to test the utility of using the "tetracotyle" type metacercariae as a biological tag was recommended. This study focused on assessing spatial and seasonal patterns of the infection of the "tetracotyle" parasite in order to test the hypothesis of multiple stocks of sardine off the coast of South Africa. A larger sample size, over an expanded area, with a longer time series was used to increase the strength of the analyses and the findings.

Spatial variation:

Spatial variation in the presence of the "tetracotyle" parasite, in sardine off the South African coast was tested and statistically analysed by comparing the prevalence, infection intensity and parasite abundance in 325 fish caught from the east and 408 fish caught from the west of Cape Agulhas. This allowed for testing the hypothesis of putative southern and western stocks of sardine.

The results obtained show that there is a clear spatial difference in the prevalence, infection intensity and abundance of "tetracotyle" type metacercariae in *Sardinops sagax* off the coast of South Africa. Both the exploratory data and the statistical analyses show that all three of these indices are significantly higher in fish to the west of Cape Agulhas and assumed to be part of the putative western stock compared to fish to the east of Cape Agulhas and assumed to be a part of the putative southern stock. A greater percentage of fish from the putative western stock are infected by the "tetracotyle" parasite, at higher infection intensities, compared to fish from the putative southern stock, all year round.

These findings are similar to those of Reed *et al.* (2012), where a difference in prevalence of "tetracotyle" type metacercariae was found between the putative western and southern stocks. However, the difference in prevalence values between fish from the putative western stock and fish from the putative southern stock was higher in the Reed *et al.* (2012) study, where prevalence ranged from 0 to 93%, with the higher prevalence values occurring in samples to the west of Cape Agulhas. This may be an artefact of sampling, whereby the sample size was greater, and the numbers of samples obtained from each putative stock were more similar in the present study.

The analysis of deviance in the Generalised Linear Model showed that stock was the most important variable in predicting the prevalence and abundance of “tetracotyle” type metacercariae, and was the second most important variable in predicting the infection intensity. In two of the analyses stock contributed the most to the observed deviance, and was the second most important contributor in the third analysis, and so is a very strong driver of the observed gradient in the distribution of “tetracotyle” type metacercariae. This confirms that there is a discontinuous distribution of “tetracotyle” type metacercariae around the South African coast, which promotes the use of this parasite as a biological tag and supports the hypothesis of the presence of multiple stocks of sardine occurring off the coast of South Africa.

Marine parasites are strongly influenced by the characteristics of their surrounding environment (MacKenzie, 2005). Distribution is primarily determined by temperature and salinity gradients of the surrounding water, and so the endemic area, where transmission of the parasite between hosts is enabled, is therefore also determined by these gradients (Timi, 2003; Timi & Poulin, 2003; Timi, 2007). In marine parasites with an indirect life cycle, such as the “tetracotyle” type metacercaria, the distribution and endemic area are further influenced by the distribution of the intermediate hosts (Janovy *et al.,* 1997; MacKenzie, 2005).

The ocean around South Africa typically has a great contrast of water masses (Branch *et al.,* 2010). Off the east coast, the prominent water movement is characterised by the Agulhas Current - a warm water, western boundary current, travelling from off the coast of Mozambique down the east coast and eventually retroflecting off the south coast and flowing eastwards (Branch *et al.,* 2010). Off the west coast, water movement is characterised by the Benguela Current - an eastern boundary current, travelling from the Southern Ocean up the west coast. In addition to this there is also prominent upwelling that takes place off the west coast due to predominant southeasterly winds blowing surface water offshore, particularly in summer (Barange *et al.,* 1999; van der Lingen *et al.,* 2006b; Branch *et al.,* 2010). Because of this, water off the west coast is typically much colder than water off the south and east coasts of South Africa. The location of the division between these two water masses is debatable due to the dynamic nature of ocean currents, however, it is thought that further offshore, the boundary between the two shifts towards Cape Agulhas (Branch *et al.,* 2010). In addition to a considerable difference in water temperature between the two environments, the catchment area of the Orange River also occurs off the west coast. The vast quantity of sediment and freshwater that flows out of this river, the largest in South Africa, significantly affects the salinity of the water off the west coast, further altering the oceanic conditions in this area (Reed *et al.,* 2012). Such differences in environmental conditions provide ideal drivers for change in the distribution of less tolerant marine species and their associated parasite assemblages (Reed *et al.,* 2012)

The large difference in environmental conditions between the west and south coasts may, directly or indirectly, explain the observed gradient in the distribution of “tetracotyle” type metacercariae in sardine around South Africa, particularly because it is thought that the division between the west and south stocks may occur near Cape Agulhas. Given that the “tetracotyle” type metacercariae abundance is much higher to the west of Cape Agulhas, the endemic area of this parasite may be off the west coast, where environmental conditions would appear to be suited to both the parasite and its first intermediate host, and thus transmission from the first to the second intermediate host is enabled. The distribution of *S. sagax*, which, as mentioned is the second intermediate host in the “tetracotyle” life cycle, appears to be much greater than that of the endemic area of the “tetracotyle” parasite. This allows for the observed gradient in “tetracotyle” prevalence, infection intensity and abundance levels. “Tetracotyle” type metacercariae were, however, not restricted to a single area either to the west or to the east of Cape Agulhas. This implies a degree of mixing between the two sardine stocks. Mixing between the putative stocks of sardine off the coast of South Africa has already been hypothesised and modelled by de Moor and Butterworth (2013), and so may explain the high parasite levels in fish to the east of Cape Agulhas.

In his study on parasites in the Argentine anchovy, *Engraulis anchoita,* Timi (2003) found a digenean metacercaria from the *Cardiocephaloides* genus to be characteristic of fish from the more southern region of the south-west Atlantic. This region is primarily influenced by the Falklands Current- a cold water, subantarctic current. The “tetracotyle” type metacercariae that is the focus of the present study is also thought to be a part of the *Cardiocephaloides* genus, and is in fact thought to be the same species, *Cardiocephaloides physalis,* that is found in the Argentine anchovy. *Cardiocephaloides physalis* has been found in the duodenum of the African penguin, *Spheniscus demersus* off the coast of South Africa. Sardine forms part of the preferred diet of this penguin, and so the occurrence of *C. physalis* in both species corresponds to what is known about the life cycle of the parasite (Horne *et al.,* 2011). According to Timi (2003) this parasite species is therefore found in colder waters, and so this supports the hypothesis that the endemic area occurs to the west of Cape Agulhas.

Because of the indirect life cycle of the “tetracotyle” type metacercariae, the distribution of the first intermediate host plays an instrumental role in the distribution and location of the parasite endemic area (Janovy *et al.,* 1997; MacKenzie, 2005). Very little is known about the life cycle of the “tetracotyle” parasite, but since digeneans infect a mollusc first intermediate host, it is presumed that the “tetracotyle” type metacercaria life-cycle involves a gastropod first intermediate host (Reed *et al.,* 2012). Osset *et al.* (2005) conducted a study on the sea bream, *Diplodus annularis* and its associated brain parasite, in the Mediterranean. The brain parasites were also in the form of “tetracotyle” type metacercariae, and occurred in aggregated clumps in the ventricles of the optic lobe of the brain, affecting the nervous system of its host. The parasite species investigated in that study was also from the genus *Cardiocephaloides,* but was instead the species *Cardiocephaloides longicollis.* The final hosts of this brain parasite are thought to be a variety of species of marine birds, while the first intermediate host is the marine gastropod *Nassarius corniculus. Nassarius corniculus* is a very common scavenging whelk that occurs in marine and brackish waters of the Mediterranean (Iannotta *et al.,* 2009).

In South African waters, the intertidal whelk, *Burnupena papyracea* may be a candidate for the first intermediate host of the "tetracotyle" parasite presumed to be *Cardiocephaloides physalis*. *Burnupena papyracea* is very abundant and occurs in high densities in the subtidal zone (Branch *et al.,* 2010). Additionally, it is a cooler water species, and is distributed to the west of Cape Agulhas up the west coast of South Africa (Orr, 1956; Branch *et al.,* 2010). The distribution of this whelk exactly fits the hypothesised endemic area of the "tetracotyle" type metacercariae. Therefore, along with the change in environmental gradient - which to a large degree also explains the distribution of the whelk itself, the presence of *B. papyracea* would explain the occurrence of the endemic area to the west of Cape Agulhas, and thus explain the higher prevalence, infection intensity and abundance of the "tetracotyle" parasite in sardine in the putative western stock.

Overall, there is a significant difference in all three population descriptors of "tetracotyle" type metacercariae in sardine between the putative western and southern stocks off the coast of South Africa, supporting the hypothesis of multiple sardine stocks. This indicates that the endemic area of the "tetracotyle" parasite is most probably to the west of Cape Agulhas, where the putative western stock of sardine had higher parasite loads. This distribution of the parasite off the west coast is most likely due to environmental gradients and the distribution of the first intermediate host, where *B. papyracea* is a probable candidate.

Temporal variation:

The overlying difference in parasite loads between the two putative stocks of *S. sagax* is complicated by the elements of interannual and seasonal variability found in the prevalence, infection intensity and abundance of "tetracotyle" type metacercariae. The exploratory data and the statistical analyses show that although fish from the western stock, overall, have much higher loads of metacercariae, there is a definite seasonal pattern in fish from both putative stocks of *S. sagax*, as well as an interannual effect. The analysis of deviance shows that season is the most important predictor of infection intensity, and after stock, is the next most important variable in predicting the prevalence and abundance of "tetracotyle" type metacercariae within individuals and within the population. Year is consistently the third most important variable in predicting the prevalence, infection intensity and abundance of the "tetracotyle" parasite. Hence season and year play a significant and important role in driving fluctuations in parasite numbers within each stock.

In the putative western stock, when normalised for year, the observed and predicted data sets show that prevalence, infection intensity and abundance all peaked in late winter. In the putative southern stock, the seasonal signal was slightly delayed, with prevalence, infection intensity and abundance peaking in spring. The minor difference in seasonal patterns shown between the putative western and southern stocks is reinforced by the fact that the analysis of deviance showed that although the season-stock interaction was the least significant predictor, it still contributed very significantly to the deviance in prevalence, infection intensity and abundance.

Previous studies have shown digeneans, both freshwater and marine, to often show seasonality in their prevalence, infection intensity and abundance within hosts (Zander & Kesting, 1998; Wang *et al.,* 2001; Harrod & Griffiths, 2005). Harrod and Griffiths (2005) conducted a study on the factors effecting the infection of pollan, *Coregonus autumnalis,* by the freshwater strigeid *Ichthyocotylurus erraticus.* That study found that seasonality played a role in the infection intensity of the parasite, where an increase was found in summer. A study conducted on the parasite fauna, many of which were digeneans, of gobiid fishes in the southwestern Baltic Sea also found seasonality in the abundances, where overall, there was an increase in the number of parasites during summer (Zander & Kesting, 1998).

Similar seasonal patterns to the ones found in the present study were found in the Argentine anchovy *Engraulis anchoita* (Timi, 2003). Prevalence and abundance in parasite loads for most parasite species examined in that study were significantly less in autumn than in spring. The pattern in the *Cardiocephaloides* species found in the eye of the anchovy was no different, where, in fact, no individuals at all were found in anchovy during autumn. Unfortunately summer and winter samples were not included in that study, limiting the full understanding of seasonality.

In the present study, the extent of the difference of parasite numbers between different seasons was not as great as was seen in the Timi (2003) study, where high prevalence and mean abundance was found in spring, while zero values for these were found in autumn. This may possibly be due to the fact that the sample sizes used in the Timi (2003) study were significantly different between seasons, being much smaller in autumn (n=44) compared to spring (n=627), whereas in the current study, sample sizes were more similar between seasons. Additionally, although both autumn and spring samples of anchovy were collected from the same area, the fish that constituted the autumn samples occupied a different zone, probably to the north of the study area, to the fish that formed the spring samples, and so seasonal driven changes in the parasite population was not the explanation for the variation in the *Cardiocephaloides* species seen between seasons.

In the present study however, the fluctuation in parasite loads between seasons and the fact that a positive relationship with length was found, suggests that the seasonal variation is driven by both changes at the fish population level, and changes at the parasite population level.

At the parasite population level, seasonality of the "tetracotyle" type metacercariae is a viable explanation for the seasonal differences in prevalence, infection intensity and abundance, in sardine. The seasonal patterns seen in the infection of *S. sagax* with "tetracotyle" type metacercariae may be because of seasonality in the distribution of the "tetracotyle" parasite within the endemic area on the west coast of South Africa. As mentioned, this depends heavily on environmental conditions and the distribution and prevalence of the parasite's first intermediate host (Janovy *et al.,* 1997; Zander & Kesting, 1998). Additionally, the release of the parasites by the first intermediate host may be driven by certain environmental changes, such as a change in water temperature. Previous studies have shown that the emergence of digenean cercariae from the first intermediate host is often related to an increase in water temperature (Wang *et al.,* 2001; Harrod & Griffiths, 2005). Seasonal patterns in oceanic conditions off the west coast are well established, with upwelling being particularly affected by season, which would thus affect water temperature (Hutchings *et al.,* 2009). In the southern Benguela, upwelling peaks during summer and thus causes a decrease in water temperature at this time of year (Hutchings *et al.,* 2009). The increase in prevalence, infection intensity and abundance of the "tetracotyle" parasite in sardine to the west of Cape Agulhas during winter may therefore be explained by an increase in water temperature at this time of year, caused by reduced upwelling, allowing for the release of the cercariae from the first intermediate host.

In addition to this, the significant positive relationships found between infection intensity and abundance of the "tetracotyle" parasite and the caudal length of its host imply that the “tetracotyle” type metacercaria accumulate in sardine, and so fish do not become uninfected by the parasite. In a study conducted by Timi and Poulin (2003) on parasite assemblages and their relation to host fish size, it was found that the characteristics of a parasite population, including the infection intensity, were positively related to length. This implies that the infections of fish by particular parasite species, such as the “tetracotyle” type metacercariae found in sardine, are, to a certain extent, not random and are relatively predictable (Timi & Poulin, 2003). The size effect is a common one in fish-parasite systems as larger fish have a higher feeding rate and can feed on larger particles, and additionally have been exposed to the parasites for longer (Timi & Poulin, 2003).

Because of the cumulative effect of the “tetracotyle” type metacercariae, and the fact that once infected, the fish cannot lose the “tetracotyle” parasites, changes at the fish population level must also occur in order to explain the reduction in prevalence, infection intensity and abundance in sardine. This is particularly the case for sardine from the putative western stock that are most likely distributed within the endemic area of the parasite. Changes at the population level may be caused by factors such as fishing and predation. These factors may result in fish being removed from the population and so would contribute to the seasonal signal that is seen in the current study. The sardine fishery primarily targets larger fish with a caudal length of approximately 20cm (de Moor & Butterworth, 2013). Due to the cumulative effect of the “tetracotyle” parasite, these fish are most likely to be the most infected, and so their removal will influence the overall parasite loads seen in each putative stock. Predators are most likely to have the same effect as the fishery and select for the larger fish, also reducing the overall parasite load seen in each putative stock.

The cumulative effect may, in part, also explain the occurrence of a seasonal signal in “tetracotyle” prevalence, infection intensity and abundance in sardine in the putative southern stock. The fact that fish cannot become uninfected by the “tetracotyle” parasite suggests that this parasite is long lived within the second intermediate host. However, since the endemic area of the “tetracotyle” parasite is thought to be on the west coast within the distribution of the putative western stock, an additional dynamic must be acting on the two stocks to explain, firstly the presence of the “tetracotyle” parasite in the putative southern stock, and furthermore, the slight seasonal pattern seen and predicted within this stock.

A shift in the host habitat will influence the distribution of the parasite assemblage (Timi & Poulin, 2003). Although two distinct stocks of sardine are hypothesised to occur off the coast of South Africa, separated at Cape Agulhas, there is likely to be considerable mixing between the two. This mixing occurs from the west to the south, when part of the western stock, particularly one year old fish, moves eastwards towards the central Agulhas Bank (Hampton, 1992; Coetzee *et al.,* 2008; de Moor & Butterworth, 2013). A model simulation conducted by de Moor and Butterworth (2013), using data from the acoustic surveys from 1985 to 2011, showed that the proportion of recruits that move from the western to the southern stock at age 1, was frequently greater than 0.6. This mixing is expected, since there are no physical barriers between the two putative stocks, and sardine are, as the sardine run shows, very capable swimmers.

If fish initially become infected with the “tetracotyle” parasite before they migrate, the influx of infected western fish would explain the slightly later peak in the “tetracotyle” parasite seen in fish in the southern stock. The peak in prevalence, infection intensity and abundance of “tetracotyle” type metacercariae in sardine off the south coast in spring is explained partly by the fact that there is a peak in parasite load in the west in winter, and partly by the fact that the spawning of sardine, although occurs year round, has an apparent peak in September to October (Barange *et al.,* 1999), and so 0 year old fish from the previous year are ready to migrate at this time of year. The cumulative effect of the parasite, implying that it is long lived within sardine, supports the occurrence of mixing between the two stocks. Fish cannot become uninfected and so will not lose the “tetracotyle” parasites before they migrate to the south coast, or when they get there.

Parasites as biological tags have been used in previous studies to show the degree of mixing between stocks and the extent of migrations, based on the assumption that if an infected fish is found outside the endemic area of the parasite, it can be assumed that the fish was once present within that endemic area (MacKenzie & Abaunza, 1998; MacKenzie, 2002). In particular, parasites as biological tags have shown to be of use in studying migrations and mixing of larger species, both pelagic and demersal. McClelland & Melendy (2011) used the parasite assemblage found on an in the Atlantic cod, *Gadus morhua* to distinguish between eastern and western stock components in the southern Gulf of St. Lawrence and the Cape Breton Shelf. Furthermore, mixing between the two components was identified during the migration and over-wintering period of the western cod. In smaller pelagic species, parasites have been used to identify migration patterns in the Pacific jack mackerel *Trachurus symmetricus murphyi* (George-Nascimento & Arancibia, 1992, as cited in MacKenzie, 2002). The use of the “tetracotyle” parasite to show not only the separation in distribution of the two putative sardine stocks, but also the mixing of the stocks, is therefore not unfounded.

Another variable may be responsible for driving the seasonal variation seen in parasite loads in this project, and that is fish age. Fish size is often used as a proxy for fish age, as fish age can be difficult to determine for large sample sizes. Although positive linear relationships were shown between caudal length and infection intensity and abundance, which is sufficient to show the cumulative effect of the “tetracotyle” parasite, looking at the graphs, the relationship shown between both infection intensity and abundance and caudal length, appears to be more exponential in nature. This may be because the accumulation of the parasites is driven more by age, than by the size of the fish. Since it was only length that was included in this study, the effect of the age of the samples may have been underestimated. If sardine off the coast of South Africa show an age-related distribution, as is suggested by Beckley and van der Lingen (1999), van der Lingen and Huggett (2003) and Coetzee *et al.* (2008), then the effect of the age of the samples may contribute significantly to the variation in parasite loads, and so this possibility needs to be considered.

Significant interannual variability, particularly in infection intensity and parasite abundance, was also found. The seasonal patterns in fish from the putative western stock were more similar in 2011 and 2012 compared to fish from the putative southern stock, and so the interannual variability was less in fish from the west of Cape Agulhas in comparison to fish from the east of Cape Agulhas. This may be explained by the fact that seasonality in the infection of fish off the west coast is primarily driven by parasite dynamics and the release of the metacercariae by the first intermediate host. Off the south coast, the seasonal signal in the infection of fish is likely to be impacted by their movement patterns rather than the parasite dynamics. This changes with year (de Moor & Butterworth, 2013), and so would explain the greater variation in seasonality seen between years in fish from the east of Cape Agulhas.

Overall, although a significant spatial signal was found in the prevalence, infection intensity and abundance of “tetracotyle” type metacercariae in fish from the putative western and southern stocks, a significant seasonal signal was also found in parasite loads in fish from both stocks. This may weaken the use of “tetracotyle” type metacercariae as a biological tag, however, knowledge of this seasonality will strongly refute this, provided seasonality is taken into account when comparing spatial differences. Drivers of the seasonal signal in the putative western stock may be at the fish population level, or at the parasite population level. The seasonal signal seen in the putative southern stock is most likely due to the significant amount of mixing that occurs between the two stocks, and therefore is as a result of the seasonal signal seen in the putative western stock. The presence of a seasonal signal in parasite prevalence, infection intensity and abundance

Limitations and assumptions:

Although conclusions from this study have been drawn there are various limitations and assumptions that need to be taken into account when interpreting the data. Firstly, because the samples from this study were obtained from commercial catches of sardine, there was a limitation in the monthly and seasonal coverage of the time series. It was difficult to obtain samples for every month from each putative stock. This was particularly the case in the summer months at the end of each year, when quotas for the year had often been reached and so no catches were made. Ideally a better monthly coverage of samples is needed, which may strengthen the seasonal analysis and allow for better interpretation of the scale at which the seasonal variance occurs. Additionally, due to time constraints, the data from this study was only collected over approximately an 18 month time period, which is not extensive enough to develop any concrete conclusions regarding interannual patterns in variability of the prevalence, infection intensity and abundance of the "tetracotyle" parasite. Because significant differences between the two years analysed were found, a longer time period is needed, so that multiple years can be compared, and more tangible reasoning for the differences can be developed. Furthermore, the sample number within each month needs to be increased in order to ensure realistic coverage and further increase the strength of the results.

Spatially, it would be of use to expand the number of localities from which samples were obtained. In the current study, samples from the west coast were mainly obtained off Gans Bay, while samples from the south coast were mainly obtained off Port Elizabeth- this was again limited by the fact that samples were obtained from commercial catches of sardine. A wider coverage of localities would be useful in strengthening the findings of this study.

During the data collection and analysis, it was assumed that the number of parasites found would not have an effect on the condition of the host. Condition could have been measured using a continuous measurement such as weight at length. However, since some of the samples were preserved in ethanol, weight was not included in the analyses, because of the effects of weight reduction due to the ethanol. Wessels (2009) in fact also found an effect of freezing on the weight of the fish, and so this could not be used to determine condition. In future studies, a measurement such as condition factor could be used, but this would have to be standardised over preservation method.

Furthermore, it was assumed that the presence of the "tetracotyle" type metacercariae in the eyes of the sardine would not affect their behaviour, to increase the likelihood of getting caught. Many previous studies have shown an alteration in host behaviour, either directly or indirectly due to the presence of a parasite. Some studies have shown an increase in predation on more parasitised hosts, particularly in invertebrates and mammals. The advantage of this is that the greater the ingestion of the intermediate hosts by the final definitive host, the greater the chance of the parasite being able to reproduce and continue its life cycle. Few studies have, however, been able to show a direct relationship between the parasite load of the intermediate host and their susceptibility to definitive host predators, in fish particularly (Barber *et al.,* 2000). La Rue *et al.* (1926), however, did report an effect on the vision of heavily infected fish by various different Strigeidae species, which may affect predator avoidance. Further research may therefore need to be conducted on behaviour changes, particularly in predator avoidance, in sardine due to the presence of the "tetracotyle" type metacercariae, in order to confirm the value of the parasite as a biological tag.

It was also assumed that the preservation method, whether it be frozen or in ethanol, would not effect the number of "tetracotyle" type metacercariae found in the sardine. Since the "tetracotyle" parasite is an internal parasite, found embedded in the eye, it was thought that this was highly unlikely and so was not corrected for in the analyses. Finally, sex was not accounted for in the analyses. Some of the fish obtained were sexually immature, and those that were preserved in ethanol were difficult to sex. Therefore, although sex was taken note of where possible, it was not included in the analyses due to the possibility of human error in sexing each specimen. The possibility of sex playing a role in the infection of a sardine by the "tetracotyle" parasite was thought to be highly unlikely.

Further research:

Because of the various limitations and assumptions of the current study, further research needs to be conducted in order to strengthen the findings and reinforce the value of "tetracotyle" type metacercariae as a successful biological tag in *S. sagax* off the coast of South Africa. As already mentioned, an insufficient time period was covered in this study in order to develop concrete conclusions about the interannual variability in "tetracotyle" type metacercariae. Further research therefore needs to focus on extending the time series. Additionally a better monthly coverage of samples from each putative stock would be useful in supporting the seasonal variation that was found.

Samples from a greater variety of localities off the coast of South Africa need to be obtained in order to strengthen the results from the spatial analysis. Samples from off the coast of KwaZulu Natal could also be added in order to further extend the use of "tetracotyle" type metacercariae as a biological tag, and possibly expand support for a third putative stock of sardine off the South African coast, although no seasonal inferences about this stock could be made due to the fact that it is only present during the winter months. The spatial analysis could also be extended to include Namibian sardine. It is known that the Namibian sardine form a discrete stock from the South African sardine, and there is a physical barrier in the form of the Luderitz upwelling cell that prevents the mixing of these two. The analysis of the presence of “tetracotyle” type metacercariae in the Namibian would therefore provide further information on the possibility of its use as a biological tag to distinguish different stocks.

Much of the interpretation of the results of this study is based on the fact that the distribution and endemic area of the “tetracotyle” parasite depends on the distribution of its first intermediate host. However, as mentioned, very little is known about this host, apart from it most likely being a type of gastropod. It is thought that this gastropod is the intertidal whelk *Burnupena papyracea,* but confirmation of this, and its distribution, is vital in supporting the presence of the endemic area to the west of Cape Agulhas and thus the use of the parasite as a biological tag. Additionally, since parasites are strongly influenced by environmental conditions, corresponding oceanic information with each sample could be of use in understanding the distribution of “tetracotyle” type metacercariae.

Finally, estimating mixing between the two putative stocks is dependent on the fact that infection occurs before the sardine migrate to the south. The hypothesis states that it is primarily one year old fish that undertake this migration, and so therefore infection by “tetracotyle” type metacercariae must take place in sardine that are under one years old. Since this study only focussed on larger, mostly adult fish obtained from the commercial catches, further research needs to also focus on juvenile sardine, in order to establish when the fish become infected. Preliminary results suggest that sardine from as early six months old become infected with the “tetracotyle” parasite (van der Lingen & Reed pers. com.), however, further research is need in order to confirm this.

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Therefore, the results from this study confirm that “tetracotyle” type metacercariae found in *S. sagax* are a good candidate for a biological tag, as suggested by Reed *et al.* (2012), where significant spatial variation is shown in its prevalence, infection intensity and abundance. However, there are a variety of limitations and assumptions that occurred in the present study that need to be taken account of. These various limitations and assumptions in turn result in further research that needs to be undertaken in order to strengthen the results of the present study.

CHAPTER 5: Conclusion

Overall, the results from this study support the previous findings of the Reed *et al.* (2012) study, and reinforce that the "tetracotyle" type metacercariae found in *S. sagax* off the coast of South Africa has potential as a biological tag. Convincing results from the prevalence, infection intensity and abundance of the "tetracotyle" parasite show that it displays high spatial disparity between the two putative stocks of sardine, to the west and to the east of Cape Agulhas, with the endemic area appearing to occur off the west coast. This therefore supports the multiple stock hypothesis, indicating that there is a separation in the sardine population off the coast of South Africa, at Cape Agulhas.

Within the endemic area, the "tetracotyle" type metacercariae do show an extent of seasonality, and so this needs to be taken into account when using the parasite to differentiate between the two putative stocks of sardine. This seasonality may occur at the population level of the parasite, and so be caused by fluctuations in the release of the parasite by the first intermediate host. In addition to this, since there is a positive relationship between the age or length of the fish and the abundance and infection intensity of the "tetracotyle" type metacercariae, a cumulative effect of infection is displayed. The cumulative effect of the infection suggests that fish cannot become uninfected by the "tetracotyle" parasite, and so the decreases in parasite prevalence, infection intensity and abundance seen between certain seasons, in the putative western stock, is also as a result of changes at the population level within the sardine population.

Having said this, a seasonal signal in "tetracotyle" prevalence, infection intensity and abundance is also shown in fish from the putative southern stock to the east of Cape Agulhas. The presence of the "tetracotyle" parasite in fish from the putative southern stock suggests that there is a reasonable amount of mixing that occurs between the two stocks, from the west to south, which reinforces what is already hypothesised. Also, the cumulative effect of the parasite means that the parasite is long-lived within the sardine, and so supports the mixing hypothesis. The delayed response in the seasonal variation in the putative southern stock, with a peak occurring in spring, supports the hypothesis that this migration to the south coast is undertaken by one-year-old recruits. The seasonal signal in "tetracotyle" prevalence, infection intensity and abundance seen in fish from the putative southern stock is therefore more likely as a result of fish movement patterns rather than parasite dynamics.

All in all, the "tetracotyle" digenean has proved to be a good biological tag to distinguish different stocks of sardine off the South African coast as it fulfils the majority of the criteria of a good biological tag. It is easily detectable and identifiable, it is not a serious pathogen causing mortality, it has high spatial disparity in infection in the different parts of the study area, and it is long-lived within the host, as shown by the cumulative effect of infection. It does not, however, have a direct life cycle, but significant advances in the understanding of the life cycle of the "tetracotyle" parasite are being made. A candidate first intermediate host has been identified, and the final vertebrate host is thought to most likely be the jackass penguin, *Spheniscus demersus*. Additionally, the seasonal patterns and interannual variability show that infection of sardine by the parasite may not be stable with time. However, knowledge and the consideration of these seasonal patterns will assist in its use as a biological tag, and effectively provide more insight into the stock structure of sardine. Ultimately, the results obtained from this study, using "tetracotyle" type metacercariae as a biological tag, suggest that there are two distinct stocks of sardine that occur to the west and east of Cape Agulhas, with mixing occurring between the two. This supports previous hypotheses of the presence of multiple stocks of *S. sagax* off our coast.

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